THE UNIVERSITY OF ALBERTA

A DIGESTIBILITY METHOD FOR RUMINANTS

by

© JOHN VAN KEULEN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

IN
Animal physiology
DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA
Spring 1977

ABSTRACT

The study involved evaluation of the acid-insoluble ash (AIA) method for measurement of digestibility of feedstuffs for ruminants. In the first part of the study the AIA laboratory analytical technique of Vogtmann et al. (1975) was examined. Modifications were made to improve the laboratory efficiency and accuracy. The modified method (2N HCl) was then compared to the AIA procedures published by Shrivastava and Talapatra [1962a (Conc. HCl)] and Vogtmann et al. [1975 (4N HCl)] in both laboratory and animal tests. An analysis done to estimate the chemical composition of AIA (2N HCl), showed that approximately 99% of AIA consisted of silica. It was also shown that AIA occurs in most common feedstuffs at readily measurable levels.

The three AIA procedures (Conc. HCl, 4N HCl and 2N HCl) were compared with the total fecal collection method using eight different rations. The results showed no difference between dry matter digestibility estimated by the three procedures as compared to the total fecal collection method. However, the digestibility estimated using the 4N HCl procedure was higher ($P<0.05$) than those estimated using the other two AIA procedures.

No diurnal excretion pattern of fecal AIA was found when 2-hourly fecal collections were made over a 24-hr period from sheep, fed a barley-oats grain ration.

Digestibility estimates obtained by the total fecal collection method from other researchers, were compared to

digestibility estimates obtained when their samples were analyzed using the 2N HCl AIA procedure. The results agreed well except for one study where the ration consisted of alfalfa and it was suspected that AIA content varied leading to errors in digestibility estimates.

ACKNOWLEDGEMENTS

I wish to thank Dr. L.P. Milligan, Chairman of Animal Science for placing the facilities of the department at my disposel.

The guidance and encouragement provided by Dr. B.A. Young, Associate Professor of Animal Physiology, are deeply appreciated and I like to thank him for his support. I also like to thank Dr. Young for editing the manuscript, which greatly benefited me.

Thanks are due to Dr. R.T. Hardin for his assistance and advice pertaining to statistical evaluations of the data.

My thanks to Dr. P.J. Martin, Alberta Department of Agriculture and Dr. R.J. Christopherson, Mr. R.D. Weisenburger and Mr. R Westra of the Department of Animal Science for use of samples for these studies.

I am thankful to Mr. J. Francis, Mr. W. Geist and Mr. N. Arbon for their technical assistance.

I am most grateful to my wife, Jenny, for her wonderful cooperation, patience and encouragement during my studies, and for her assistance in typing this thesis. I also thank my children, Carla, Jerry, Allan and Heather for helping me in many important ways.

Financial support for this project from the National Research Council of Canada and the Alberta Agriculture Research Trust was appreciated.

TABLE OF CONTENTS

Section PAGE

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 6

A. Digestibility by Fecal Index Method 7

B. Digestibility by Ratio Method 8

C. External Index Substances 9

1. Chromic oxide 9
2. Polyethylene glycol 10
3. Polyethylene 11

D. Internal Index Substances 11

1. Lignin 11
2. Plant chromogen 12
3. Fecal nitrogen 13
4. Silica 13
5. Acid-insoluble ash 16

III. EXPERIMENTAL 18

A. Introduction 18

B. Examination of Individual Steps in 4N HCl Laboratory Procedures 19

Test 1. Acid Strength 19
Test 2. Filtering Efficiency 20
Test 3. Time of Acid Digestion 21
Test 4. Boiling Time 22
Test 5. Hot Plate Temperature and Acid Strength 23
Test 6. Washwater Temperature 24

C. Initial Modifications to the 4N HCl Laboratory Procedure and their Evaluations 26

Test 7. Ashing Prior to Acid Treatment .. 27
Test 8. Acid Strength 28
Test 9. Acid Volume 29

SECTION PAGE

Test 10. Acid Volume 30
Test 11. Boiling Temperature 31
Test 12. Washwater Temperature 32
Test 13. Furnace Differences 33
Test 14. Acid Strength and Sample Size .. 34

D. Second Modifications and their Evaluations 36

Test 15. First Ashing Temperature 37
Test 16. Second Ashing Temperature 40
Discussion of Laboratory Procedures 42

E. Furnace Capacity 47

F. Composition of AIA 52

G. AIA Content of Common Feedstuffs 54

H. Comparison of Three AIA Laboratory Procedures 60

I. Comparison of AIA Methods Using Animal Feeding Trials 67

Experiment 1. Evaluation of AIA as a Natural Index Substance in Ruminant Digestibility Studies 67

a. Experimental design, animal management and rations 67
b. Feed and fecal collections 69
c. Analysis and calculations 70
d. Statistical analysis 71
e. Results 71
f. Discussion of Experiment 1 77

Experiment 2. Comparison Between the 2N HCl and Total Collection Methods in Estimating Digestibility by Sheep 80

a. Experimental design, animal management and rations 80
b. Analysis 81
c. Results 81
d. Discussion of Experiment 2 85

SECTION PAGE

J. Experiment 3. Diurnal Variation 87

1. Experimental 87
2. Results and discussion 98

K. Comparison between Total Collection and AIA Estimated Coefficients of Digestibility 93

Study A. 93
Study B. 95
Study C. 95

IV. GENERAL DISCUSSION 100

BIBLIOGRAPHY 106

APPENDIX 114

LIST OF TABLES

Table Page

1. Mean (±SE) AIA values of brome grass using different normalities of HCl acid 20
2. AIA contents (%) of brome grass using different acid digestion times (pre-boiling) and effect of boiling 22
3. Mean (±SE) AIA values in brome grass using different boiling times 23
4. Analysis of variance of the effects of hot plate temperature and normality (2N and 4N) on the AIA content of brome grass 24
5. Mean AIA values (%) of brome grass using different hot plate temperatures and two normalities (2N and 4N) of HCl acid 25
6. Mean AIA values (%) of brome grass when different washwater temperatures and two acid normalities were used 26
7. Mean (±SE) AIA values of brome grass using different normalities of HCl acid 29
8. Mean (±SE) AIA values of brome grass using different volumes of 4N HCl acid 30
9. Mean (±SE) AIA values of brome grass using different acid temperatures 31
10. Mean (±SE) AIA values of brome grass using different boiling times 32
11. Mean (±SE) AIA values of brome grass using different washwater temperatures 33
12. Mean (±SE) AIA values of brome grass obtained by using four different furnaces 34
13. Mean AIA values (%) of brome grass and feces determined by using different normalities of HCl and two fecal sample sizes 35
14. Mean AIA values of brome grass, concentrate and feces using different first ashing temperatures. 38

Table Page

15. Mean AIA values for brome grass, concentrate and feces by using different second ashing temperatures 42

16. The AIA content of common feedstuffs determined by the 2N HCl method 57

17. Estimation of (a) laboratory occupancy time (requiring presence of operator) and (b) laboratory time (not requiring presence of operator) required to analyze 12 samples by the three (Conc. HCl, 4N HCl and 2N HCl) AIA laboratory procedures 63

18. General observations and a comparison between the Conc. HCl, 4N HCl and 2N HCl laboratory procedures on the basis of materials (per sample) required 66

19. Percentage composition of rations 68

20. Percentage AIA in rations determined by three AIA methods 72

21. Relationships between dry matter digestibility coefficients (%) determined by the total collection (TC), 4N HCl and 2N HCl methods 72

22. Analysis of variance of the effects of rations and AIA methods on estimation of dry matter digestibility coefficients (%) 75

23. Mean dry matter digestibility coefficients (%) of rations (Table 19) estimated by total collection and three AIA methods. Two animals were used for each ration 75

24. Mean recovery (%) in feces of AIA. Two sheep were used for each ration. AIA content in feed and feces was determined by three AIA methods ... 76

25. Mean percent digestibility of dry matter and of energy on dry matter basis for three rations estimated by the total collection (TC) and 2N HCl methods 82

26. Percent AIA of alfalfa (ration 3) on individual days during three 8-day collection periods 84

Table Page

27. The AIA values of fecal samples collected at 2-hourly intervals during a 24 hour period and estimated by the 2N HCl method 89

28. AIA values of fecal samples collected at 2-hourly intervals during a 24-hour period, as determined by the 2N HCl method and presented as a percentage when the mean AIA value of each animal was expressed as 100 90

29. AIA recovery and dry matter digestibility coefficients estimated by the total collection (Westra, 1975) and the 2N HCl methods 94

30. AIA recovery and dry matter digestibility coefficients estimated by the total collection (Weisenburger, 1976) and the 2N HCl methods 96

31. AIA recovery and digestibility coefficients estimated by the total collection (Christopherson, 1976) and the 2N HCl methods ... 97

LIST OF FIGURES

Figure Page

1. Acid-insoluble ash analytical procedures 20

2. AIA contents of (a) swine ration, (b) brome grass and (c) feces from sheep fed brome grass, estimated by using different first ashing temperatures 39

3. AIA contents of (a) swine ration, (b) brome grass and feces from sheep fed brome grass, estimated by using different second ashing temperatures 41

4. Schematic representation of incomplete combustion 49

5. Relationships between dry matter digestibility coefficients from three AIA methods (Conc. HCl, 4N HCl and 2N HCl) and the total collection method 73

6. Dry matter digestibility coefficients of a barley-oat grain ration, estimated by the 2N HCl method from samples of feces taken at 2-hourly intervals during a 24-hr period. Each line represents results from one sheep 92

I. *INTRODUCTION*

A major portion of the cost of animal production is the feed cost. Consequently, if a livestock operation is to maximize profit, it is most essential that particular attention be given to achievement of an economical feeding system. Only with knowledge of the nutritional requirements of the animals and the nutritive values of the feedstuffs, can one hope to attain maximum efficiency in animal production. In order to obtain nutritive values of feedstuffs the need for feed evaluation becomes apparent.

Different methods are being employed for the evaluation of feedstuffs. Visual examination, although widely used and the only practical method when an immediate evaluation is required, does not provide any information about the availability of energy and nutrients in the feed. Determination of the chemical composition (proximate analysis) of a feedstuff is the starting point for the estimation of the nutrient value. However, the quality of a ration cannot be accurately and fully ascertained by chemical analyses alone (Crampton, 1950; Raymond, 1951). Despite the hesitation, chemical analyses plays an important role when used in conjunction with animal trials (Van Soest, 1969).

Apparent digestibility is a nutritional term used to describe the proportion of a feedstuff which, when ingested by an animal, does not appear in the feces. The qualification 'apparent' is used because some substances appearing in the feces arise endogenously and are not directly

associated with the ingested feedstuff. Because of the frequent use in this thesis the qualification is omitted and simply the word 'digestibility' is used. Furthermore, unless specified the word 'digestibility' is used to refer to the dry matter (DM) portion of the feedstuff.

A direct estimate of the digestibility can be obtained from measurements of the quantity of material ingested and the quantity of feces excreted (Crampton, 1950; Kane et al., 1953; McDonald et al., 1969). This is the total collection method. To ensure complete fecal collection it is necessary to confine animals in individual crates or cages for several days or to attach harnesses and bags for the collection (Garrigus, 1934). Such confinement of the animals may lead to stress and affect digestibility values (Ellenberger and Schneider, 1927; Waldo et al., 1961; Noblitt et al., 1963; Phar et al., 1971). Because of the restraint and possible stress on the animal and because of the inconvenience and time involvement in total collection studies, simplier and more convenient means for estimation of digestibility have been sought.

Most indirect methods for estimating digestibility are based on the measurements of a reference substance in the feed and/or feces (Schneider et al., 1955). Several synonyms (index, indicator, marker) are used in the scientific literature to describe these reference substances. Kotb and Luckey (1972) have classified them into two main groups: (1) absorbable and (2) non-absorbable. The second

group contained those most commonly used in digestibility studies. These substances may be further classified into two main groups: (1) external index substances, substances which are added to a feedstuff or taken orally such as chromic oxide and (2) internal index substances, substances which occur naturally in a feedstuff such as lignin.

Kotb and Luckey (1972) extensively reviewed and evaluated the use of many different index substances. One index substance that has been suggested but not extensively evaluated is the acid-insoluble ash (AIA) or residue of feedstuffs. In fact, this index substance was overlooked by Kotb and Luckey in their review.

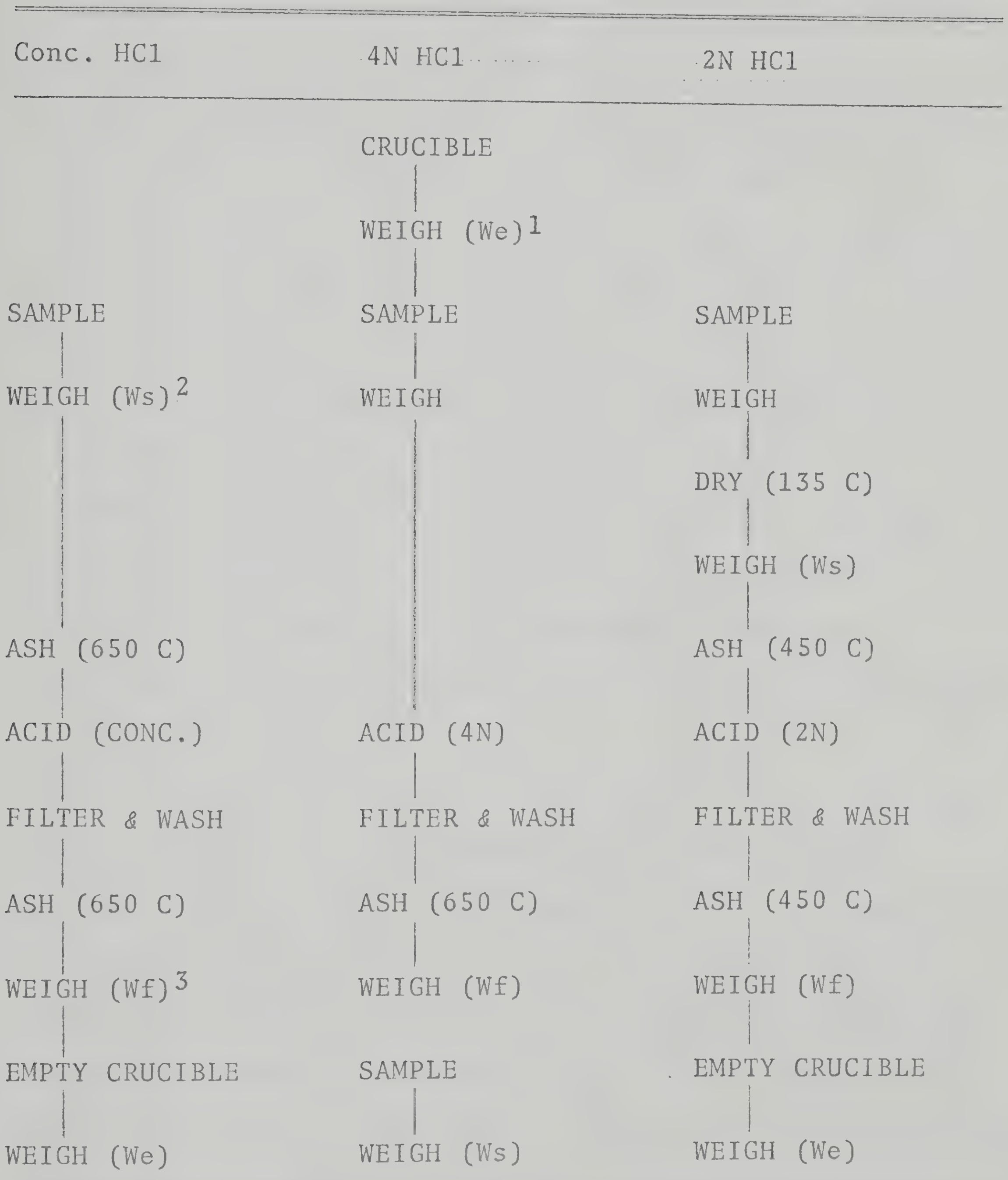
The use of an acid-insoluble residue of feed was first reported by Shrivastava and Talapatra (1962a) when used to determine the digestibility of feeds by sheep. Results obtained by Shrivastava and Talapatra were encouraging and closely approximated the results obtained from the total collection method. McCarthy et al. (1974) used AIA as an index substance to estimate the digestibility of swine rations and also obtained satisfactory results with this index substance when compared with the total collection method. Vogtmann et al. (1975) compared the metabolizibilty of fatty acids in poultry rations, determined by means of the AIA index substance, to results obtained by the total collection method and concluded that AIA was a suitable index substance for determination of the digestibility of poultry rations. These findings led to the present study

in which AIA was evaluated for use as a digestibility index substance for ruminant rations.

Because of the empirical nature of previous studies evaluating AIA, the first part of the present study involved examination and evaluation of the AIA laboratory analytical technique of Vogtmann et al. (1975), which was the same as that used by McCarthy et al. (1974). On the basis of those results some modifications were made to improve the laboratory efficiency and accuracy. Tests were made to evaluate the new procedure in both laboratory and animal tests against the procedures published by Shrivastava and Talapatra (1962a), Vogtmann et al. (1975) and the total collection method. Further tests were then conducted to establish the reliability of the modified method and to determine if there were diurnal variation excretion patterns of AIA in the feces.

The term AIA used in this study, represents the insoluble ash or residue left over when samples (usually feed or feces) are digested with hydrochloric acid (HCl) and ashed. Ashing sometimes proceded the acid digestion. The three basic laboratory techniques for AIA, often referred to in the thesis are referred to simply in terms of the concentration of HCl acid used during the acid digestion, viz., Conc. HCl (Concentrated HCl), 4N HCl and 2N HCl. Stepwise descriptions, laboratory analytical procedures of the three basic AIA methods are given in Appendix 1, 2 and 3 and a schematic summary of each is contained in Figure 1.

Figure 1. Acid-insoluble ash analytical procedures. Details of each are given in Appendices 1, 2 and 3.



---

[1] Wf = Weight of crucible with ash, in g.

[2] We = Weight of empty crucible, in g.

[3] Ws = Weight of sample, in g dry matter.

II REVIEW OF LITERATURE

Kotb and Luckey (1972) suggested that before an index substance qualifies as an effective nutritional index substance it should: (1) be inert with no toxic physiological effects; (2) be neither absorbed nor metabolized within the gastrointestinal tract; (3) have no appreciable bulk; (4) mix intimately with and remain uniformly distributed in the digesta; (5) have no influence on gastrointestinal secretion, digestion, absorption, motility or excretion; (6) have no influence on the microflora of the gastrointestinal tract; and (7) have physical - chemical properties which allow ready and precise quantitative measurements. However, as pointed out by Engelhardt et al. (1974), none of the available index substances satisfy all these criteria.

The application of indirect methods for estimating forage intake by grazing animals have been, recently, reviewed by McDonald (1968), Raymond (1969), Streeter (1969) and Kotb and Luckey (1972). Schneider and Flatt (1975) have also reviewed the overall methodology of feedstuff evaluation. The present review is focussed on the use of two indirect methods for estimating digestibility of feedstuffs and considers several external and internal index substances used in digestibility studies.

A. *Digestibility by Fecal Index Method*

This method has been used mainly with grazing ruminants and was developed by researchers who questioned the ability of obtaining representative forage samples by hand or mechanical clipping (Raymond et al., 1954). Digestibility is estimated by: (1) using the total collection method to obtain digestibility coefficients from cut forages fed to penned animals; (2) establishing by regression equation the best relationship between the fecal index substance and the digestibility coefficients of the pen fed forages; (3) collecting fecal samples from grazing animals; and (4) substituting the fecal index substance concentration into the previously established regression equation and estimate the digestibility. Unlike the ratio method, (see below) index substances used in the fecal index method need not be completely indigestible (Kotb and Luckey, 1972).

Some of the fecal index substances that have been proposed include chromogen (Reid et al., 1952), nitrogen (Lancaster, 1949) methoxyl (Richards and Reid, 1952) and crude fiber (Raymond et al., 1954). Fecal nitrogen and chromogen were used extensively and considered more reliable than the others (Kotb and Luckey, 1972).

The main difficulty with the use of the fecal index method is obtaining a representative sample of the forage consumed by the animal (McDonald, 1968). Diurnal variations of the index substance excretion rates (Kotb and Luckey, 1972) and high labor requirements (Langlands, 1975) are also

serious limitations to the use of this method. To improve forage sampling, esophageal fistulated animals have been used (Van Dyne and Torrell, 1964). Selected fecal sampling schedules were adopted to overcome diurnal variations of index substance concentration (Smith and Reid, 1955). Both the above improvements increase the labor requirements.

B. *Digestibility by Ratio Method*

By the ratio method an estimate of digestibility is obtained from the ratio of concentration of the index substance in the feed and feces (Kleiber, 1975).

$$\text{Digestibility (\%)} = 100 - 100\,\frac{Mi \times No}{Mo \times Ni}$$

where

Mi = % index substance in the feed.

Mo = % index substance in the feces.

Ni = % nutrient in the feed.

No = % nutrient in the feces.

The validity of the use of the ratio method is based on the ability to obtain representative samples of the feedstuff consumed and feces produced and on the assumption that the index substance is inert and passes through the alimentary tract at a uniform rate. Diurnal variation in the concentration of the index substance in the feces could lead to biased and/or variable estimates of digestibility.

C. External Index Substances

1. Chromic oxide

Chromium sesquioxide (Cr_2O_3) is presently the most commonly used index substance in nutrition studies. However, its use has not been wholly satisfactory. Incomplete and inconsistant recoveries have been reported frequently (Smith and Reid, 1955; Davis et al., 1958; Elam et al., 1959; Carew, 1973; McCarthy et al., 1974). Diurnal variations have been demonstrated to be quite large, especially when the index substance is given once daily (Brisson et al., 1957). Brisson and co-workers, were however, able to reduce diurnal variation by administering the chromic oxide in a pelleted form, six times daily. The chromic oxide pellet (Pigden and Brisson, 1957) and paper impregnated forms (Corbett et al., 1958) were developed in attempts to minimize variation in its excretion pattern. The labor and frequent restraining of the animals limits the use of this approach and substancially reduces the advantage the ratio method might have over the total collection method.

Kiesling et al., (1969) reported that the incorporation of chromic oxide into shredded paper, fed once daily to grazing steers, produced recoveries considerably less than 100% and was highly variable among steers. These authors concluded that administering the index substance in impregnated paper was no better than other methods of administration.

MacRae (1974) reported that chromic oxide does not associate itself with the solid phase of digesta which raises the question of its use in studies where a solid feed index substance is required.

2. *Polyethylene glycol*

Polyethylene glycol (PEG) is widely used as a total digesta and liquid-phase index substance (MacRae, 1974), however, some researchers have used it as a water-soluble index substance to estimate feedstuff digestibility (Sperber et al., 1953; Corbett et al., 1958). Corbett et al. (1956) reported its suitability as an index substance for estimating fecal output in cows. Sperber et al. (1953) showed a PEG recovery of 90% in feces and found no appreciable absorption or destruction in the digestive tract. They inferred that digestion studies should be possible using PEG but they did not specifically report data showing the accuracy of such determinations. Sinha et al. (1970) studied the feasibility of using PEG as an index substance to estimate digestibility of feedstuffs in cattle. They reported a low regression coefficient ($r = 0.008$) between the estimated digestibility using PEG and the digestibility estimated by the total collection method. On the basis of the obtained results, Sinha and co-workers questioned the use of PEG in digestibility studies.

It appeared that PEG associates itself with the liquid phase and not with the solid phase of digesta. In addition,

the use of PEG as an index substance is limited because of the lack of a readily available accurate and sensitive analysis method.

3. Polyethylene

Limited but satisfactory results were obtained by Chandler et al. (1964, 1966) when they used microthene polyethylene powder as an index substance to estimate dry matter digestibility in calf rations. Their results showed a 100% recovery of the index substance with no evidence of a diurnal variation. This index substance, however, has not been evaluated for estimation of digestibility with older animals.

D. Internal Index Substances

1. Lignin

In 1946, Ellis et al. (1946) suggested lignin as an index substance for estimating digestibility because it seemed to meet the conditions of an ideal index substance. However, there has been some controversy over the validity of considering lignin an indigestible plant constituent (Bondi and Meyer, 1948). Smith et al. (1956) showed that from 2 to 11% on lignin of lucerne hay was digested by deer and from 9 to 15% by sheep.

Kane et al. (1951) reported the results obtained using the lignin method and it appeared that the results were influenced by the type of plants used and by the presence

of a diurnal variation in the concentration of lignin in feces. This variation meant that fecal samples had to be obtained at different intervals of the day and night to obtain a representative daily sample. Furthermore, variation in digestibility and recovery may be caused by the analytical methods used for lignin (Van Soest, 1964).

2. *Plant chromogen*

The use of the plant chromogen as an internal index substance for estimation of digestibility was suggested by Reid et al. (1950). This method employed plant pigments that absorbed light at 406 mμ and were found to be indigestible and completely recovered in feces. A spectroscopic examination of an 80% acetone extract of the feedstuffs and feces revealed these substances. In a subsequent paper Reid et al. (1952) reported that feedstuffs low in chromogen compounds (hay and concentrate) gave an incomplete recovery of chromogen in the feces. Furthermore, significant errors were found with feedstuffs containing very large or very small quantities of chromogen. The same authors, however, developed a mathematical relationship between the chromogen-dry matter ratio of feces voided and that of feedstuffs consumed.

From studies comparing lignin and chromogen as index substances for estimation of consumption and digestibility of forage in sheep, it was concluded by Cook and Harris (1951) that chromogen was satisfactory for lucerne but not

suitable for estimating digestibility of winter range forage. Greenhalgh and Corbett (1960) reported a greater variation in estimated digestibility with chromogen than when nitrogen was used.

3. Fecal nitrogen

Fecal nitrogen (undigested, endogenous and indigestible protein) is correlated with dry matter intake (Blaxter and Mitchell, 1948), a fact that is explained when it is realized that a significant portion of the fecal nitrogen is endogenous.

Lancaster (1949) showed that fecal nitrogen concentration was related to the digestibility of forages and developed regressions (Lancaster, 1954) relating fecal nitrogen to digestibility.

When fecal nitrogen was used by Holter and Reid (1959), it appeared to be suitable for estimation of dry matter digestibility in forages, grazed by ruminants. However, Minson and Kemp (1961) reported large errors associated with the equations in which digestibility was correlated with the nitrogen content of feedstuff and feces.

4. Silica

The use of silica as an internal index substance for determining digestibility was introduced by Wildt in 1874 (quoted by Kotb and Luckey, 1972). Silica was suggested, because it occurred naturally in the feed and was apparently

not absorbed by the animal. The reports on accuracy of the method have been diverse, some being distinctly favorable (Gallup, 1929; Gallup and Kuhlman, 1931; Jones and Handreck, 1965a), while others suggested a limited applicability (Gallup and Kuhlman, 1936: Gallup, Hobbs and Briggs, 1945).

When silica and lignin were used to estimate forage intake with grazing ruminants, the results calculated from the use of silica showed more variation than those calculated from the use of lignin (Van Dyne and Meyer, 1964). The main problems encountered were the non-quantitative recovery of silica in the feces and contamination of the feed and feces with either soil or dust. Two factors suggested as the reasons for the incomplete recovery of silica were contamination of the feed with dust and the absorption of silica from the digestive tract and its excretion in the urine (Forman and Sauer, 1962). Jones and Handreck (1965a) showed that the solid silica of dry plant feeds ingested by sheep can be completely recovered in the feces and urine.

Animals ingest appreciable amounts of silica which is present in plants as soluble and insoluble silica (Jones and Handreck, 1965b). Silica in soil solution is undissociated monosilicic acid and is absorbed through simple solution in the transpiration stream. The monosilicic acid is carried to the tops (stems) of the plant and polymerizes to form solid silica as water is lost by transpiration (Handreck and Jones, 1968). It is deposited therefore, in greatest quantities in those parts and regions from which

water is lost in greatest quantities. The silica contained in several grasses was identified as opal ($SiO_2.nH_2O$) (Parry and Smithson, 1957; Smithson, 1958).

Daily intake of silica by ruminants varies with feed intake, the individual animal and the silica content of the plants consumed. Sheep, grazing pasture of barley which contained at three different stages of growth 1.70, 2.81 and 3.65 percent of silica, excreted silica in daily amounts of 6.2, 14.7 mg and 20.6 g in their feces and 164, 95.3 and 76.2 mg in their urine, respectively, (Nottle and Armstrong, 1966).

Monosilicic acid was found in the recticulo-rumen fluid of sheep (Jones and Handreck, 1965a) and cattle (Bailey, 1976). This meant that there is some dissolution of ingested silica which levels off at approximately 190 ppm of monosilicic acid in sheep and slightly above 200 ppm in cattle reticulo-rumen fluid. When the feed contained more than two percent silica the reticulo-rumen fluid was saturated (Jones and Handreck, 1965a). The concentration of monosilicic acid in the reticulo-rumen of cows was also shown to level off at, or slightly above, the saturation concentration of 200 ppm, at all levels of intake when a rough fescue (*Festuca scrabrella*) ration (5.70% silica) was used (Bailey, 1976). The absorbed monosilicic acid is carried in the blood stream to the kidneys (Jones and Handreck, 1965a). Its concentration in the blood stream remained constant in sheep and cattle with different levels of intake. The amount of monosilicic acid

excreted in the urine was influenced by the ration and amount of water consumed. In sheep the urinary excretion of silica increased with increasing silica intakes of up to eight gram per day, but no further increases followed from increased intakes and excretion plateaued around 200 mg of silica per day (Nottle, 1966). In cattle the excretion rate for monosilicic acid was nearly constant over extended periods (Bailey, 1967).

Jones and Handreck (1965a) estimated the DM digestibility coefficients of three rations using silica and found the results to be in close agreeement with those determined by the total collection method. Furthermore, they showed that practically all of the silica was excreted in the feces and on the bases of their results suggested that silica be re-examined as an indicator for digestibility trials.

5. *Acid-insoluble ash*

The use of AIA of feedstuff and feces as an index substance for determination of digestibility for ruminant feedstuffs was first reported by Shrivastava and Talapatra (1962a). Their findings indicated an average recovery of the residue to be 99.8% (91.2 - 108.7%) and the estimated digestibility coefficients were, from a practical standpoint, not different from values obtained by the total collection method. The same authors used this index substance to determine the pasture consumption and level of nutrition of grazing sheep (Shrivastava and Talapatra, 1962b). They

obtained average values for dry matter consumption per 45 kg body weights of 1.18 kg for both pen-fed and grazing animals.

Recently, McCarthy et al. (1974) evaluated the use of AIA as an index substance for determining digestibility of rations in growing pigs and concluded that the 4N HCl method as described by Vogtmann et al. (1975) was superior to that of using chromic oxide as an index substance in swine diets. Furthermore, Vogtmann et al. (1975) concluded that the 4N HCl method gave a similar accuracy to the total collection method for determining metabolizability of enery and digestibility of fatty acids in broiler diets.

On the basis of the above results, it appears that AIA has potential as an index substance for digestibility studies. However, various aspects of AIA such as sensitivity of steps in the laboratory procedure, composition, use in ruminant digestibility studies and the determination of its level in common feedstuffs, require further studies.

III EXPERIMENTAL

A. Introduction

The 4N HCl AIA method has been described for digestibility studies in swine (Mc Carthy et al., 1974) and poultry (Vogtmann et al., 1975). In the present study this method was examined for determining digestibility of rations by sheep.

In view of the empirical nature of the laboratory procedure and the unknown significance of each step to the overall accuracy of the method, it was decided to examine the laboratory procedure first to determine which steps were most critical and make changes where necessary. In addition, it was suggested (John McCarthy, personal communications) that some steps of the laboratory procedure could be trobelesome. For example, the odor and fumes created when feed or fecal samples were boiled in 4N HCl acid required an efficient fumehood. However, working in a fumehood restricted the size and type of apparatus that could be used. After extensive laboratory evaluation and revision the method was applied and evaluated in animal feeding trials.

All AIA sample calculations were done to four decimal places in order to avoid round-off error. Accordingly, AIA values presented in sections B, C and D have all been calculated to four decimal places before being rounded off to three decimal places for presentation. Duncan's New Multiple Range Test (Steel and Torrie, 1960) was used in

comparison of means. Analysis of variance was done with computer programs available from the University of Alberta Computing Centre.

B. Examination of Individual Steps in the 4N HCl Laboratory Procedure.

During these examinations of the 4N HCl method the laboratory procedures described by Vogtmann et al. (1975) were followed except for the step being tested. An outline of Vogtmann's laboratory procedure is contained in Appendix 2.

Except were indicated the laboratory tests were on samples of brome grass (IRN: 1-00-890)[1] dried to constant weight in a forced air oven and finely ground in a Christy-Norris mill[2].

Test 1. Acid Strength

Duplicate brome grass samples were analyzed for AIA content using acid normalities of 0.5, 1, 2, 3, 4 and 5N HCl instead of the standard 4N HCl.

The 6N HCl was rejected because: (1) the filtrate boiled up into the steam condensor and HCl fumes escaped; (2) a particular obnoxious and irritating odor was produced during the digestion; (3) the filter paper tended to break during the filtering process; (4) black residue remained

---

1. International Reference Number.
2. Model No. 8, Chelmsford, England.

Table 1. Mean (±SE) AIA values of brome grass using different normalities of HCl acid.

Normality of HCl	AIA value (%)[1]	SE
0.5	3.010^{c}	0.024
1.0	2.905^{ab}	0.015
2.0	2.878^{a}	0.007
3.0	2.924^{ab}	0.002
4.0	2.940^{b}	0.014
5.0	2.993^{c}	0.016

[1] SE of means 0.015%.
[a-c] Means with different superscripts are significantly ($P<0.05$) different.

adhered to the walls of the flask after washing. From the analysis of variance of the results for 0.5 to 5N acid a significant ($P<0.01$) effect of acid strength was indicated (Table 1). The 2N acid gave the lowest AIA value but was not significantly different from the 1N and 3N HCl values.

Test 2. Filtering Efficiency

While further tests with 6N HCl were discontinued for the above reasons, the question of effect of the acid, at least to a strength of 5N HCl, on the filter paper remained unsolved. Twelve feed samples were, therefore, analyzed by the 4N HCl procedure but 100 ml of 5N HCl was passed through the filter paper immediately prior to the filtering step. These results were then compared with values obtained when 100 ml of water was passed through the paper immediately prior to the filtering step.

The mean (±SE) AIA contents of brome grass from the acid-treated and non-treated filterpapers were 2.944 ± 0.018% and 2.942 ± 0.014%, respectively. It was therefore, concluded that 5N HCl did not alter the filtering efficiency of Whatman No 41 filter paper.

Test 3. Time of Acid Digestion

The duration of cold digestion was defined as the length of time between the addition of acid at room temperature to the sample, till the mixture (sample plus acid) was placed on the hotplate. Two tests were made. In the first test, samples (six replications) were cold digested for 30 and 60 min. A further six samples were cold digested for one min but not boiled in acid. In the second test, duplicate samples were cold digested for 1, 15, 30, 60, 90, 150 and 240 min. Half the samples in the second test were then boiled for the standard 30 min while the boiling step was omitted for the other samples. In both tests, the acid was added to the samples at different times so that samples were processed together for the final steps. The one minute cold digestion time in both tests, represented the time required to add acid to the samples before they were either washed free of acid or boiled in acid.

In the first test the mean (±SE) AIA content of brome grass samples were 3.163 ± 0.006% and 2.884 ± 0.010% for the 30 and 60 min cold digestion periods, respectively. The 30 and 60 min cold digestion treatments were not

Table 2. AIA contents (%) of brome grass using different acid digestion times (pre-boiling) and effect of boiling.

Acid temperature	Acid digestion time (min)						
	1	15	30	60	90	150	240
Boiled[1]	2.786	2.837	2.804	2.795	2.774	2.809	2.805
Non-Boiled[2]	3.166	3.108	3.134	3.107	3.105	3.017	3.128

[1] Mean (±SE) is 2.801 ± 0.008%.
[2] Mean (±SE) is 3.109 ± 0.017%.

significantly different, but both treatments resulted in significantly ($P<0.05$) lower AIA values than non-boiled samples.

The second test, also showed significantly ($P<0.001$) higher values for the non-boiled samples, however, cold digestion time from 1 to 240 min had no effect on AIA content (Table 2).

Test 4. Boiling Time

To determine the effect of boiling time on AIA content, feed samples (four replications) were boiled for 15, 30 and 60 min, instead of the normal 30 min time period.

Increasing the boiling time appeared to result in an increased estimated AIA content of brome grass (Table 3). However, no significant difference between the AIA values of the 15 and 30 min boiling times was obtained and in turn, AIA values from the 30 and 60 min boiling times were not significantly different. Therefore, it appeared that a

Table 3. Mean (±SE) AIA values of brome grass using different boiling times.

Boiling time (min)	AIA value (%)[1]	SE
15	2.844[a]	0.005
30	2.867[ab]	0.022
60	2.923[b]	0.010

[1] SE of means 0.014%.

[a-b] Means with different superscripts are significantly ($P<0.05$) different.

30 min boiling time may be reduced to 15 min without a significant change in AIA values.

Test 5. Hot Plate Temperature and Acid Strength

The use of a hot plate for heating the sample mixtures during acid digestion resulted in some samples being boiled more vigorously than others, due to uneven heat distribution from the hot plate surface. A test was, therefore, conducted in which duplicate samples were boiled on different hot plates, using different temperature settings. Six temperatures of approximately 100, 110, 120, 130, 150 and 160 C were used with two normalities of HCl (2 and 4N). The 2N HCl was used to check the results obtained in Test 1. A thin layer of sand, containing a mercury thermometer, was placed on top of the hot plate to measure the effective heat coming from the hot plate. The Erlemeyer flask, containing the sample mixture, was then placed on top of the sand.

Table 4. Analysis of variance of the effects of hot plate temperature and normality (2N and 4N) on the AIA content of brome grass.

Source	Degrees of freedom	Mean Square	F-value
Normality	1	0.00258	8.32*
Temperature	5	0.00815	26.29***
Interaction	5	0.00118	3.81*
Error	12	0.00031	
Total	23		

*P<0.05
*** P<0.001

The effects of temperature, normality and interaction between temperature and normality on the AIA content of brome grass were all significant (Table 4). The mean AIA values for the 2N HCl and 4N HCl used were 2.797 and 2.817% respectively. The mean results presented in Table 5 indicate that a temperature of 130 C, or lower, resulted in AIA values which were significantly (P<0.05) higher than those boiled using higher hot plate temperatures.

The use of 2N HCl resulted in lower (P<0.05) AIA values than 4N HCl. Thus confirming the Test 1 observations on the effect of acid strength.

Test 6. Washwater Temperature

In the Vogtmann et al. (1975) publication of the 4N HCl method, washwater temperature was not specified, only referred to as: "hot distilled water". A test was made to

Table 5. Mean AIA values (%) of brome grass using different hot plate temperatures and two normalities (2N and 4N) of HCl acid.

Acid strength	Temperature of hot plate (C)						SE of means
	100	110	120	130	150	160	
2N	2.858	2.820	2.858	2.778	2.726	2.740	0.012
4N	2.850	2.865	2.822	2.818	2.770	2.780	0.013
Mean	2.854^c	2.842^c	2.840^c	2.798^b	2.748^a	2.760^a	0.009

[a-c] Means with different superscripts are significantly ($P<0.05$) different.

determine if washwater temperature had an effect on AIA content. Duplicate samples were analyzed using washwater temperatures of approximately 25, 50, 75 and 100 C and two normalities of HCl (2N and 4N) were used.

An analysis of variance of the results showed no significant difference in AIA content between washwater temperatures when either 2N or 4N HCl were used (Table 6) The mean AIA values from the 2N and 4N HCl (2.838 and 2.929%, respectively) were again significantly ($P<0.001$) different.

From this test it was concluded that washwater temperature was not influencing the AIA value.

Table 6. Mean AIA values (%) of brome grass when different washwater temperatures and two acid normalities were used.

Acid strength	Washwater temperature (C) 25	50	75	100	SE of means
2N	2.842	2.851	2.830	2.828	0.005
4N	2.916	2.937	2.934	2.930	0.007

C. *Initial Modifications to the 4N HCl Laboratory Procedure and their Evaluations*

Presented in this section are the initial modifications made to the 4N HCl method (Appendix 2). Previously all acid digestions for AIA analysis in our laboratory were done on a hot plate in a fumehood to prevent the rather unpleasant odor from spreading throughout the laboratory area. This procedure, however, created additional problems. The heat from a hot plate was not evenly distributed and resulted in uneven boiling of some samples on the plates. Furthermore, each steam condenser had to be closely watched and adjusted frequently to prevent HCl from boiling off.

Many tests were undertaken to ascertain suitable modifications and to evaluate the modifications. The more critical of the tests which lead to modifications are presented herein, but not necessarily in the order in which they were taken. Tests on all permutations and combinations of modifications were not possible. Consequently,

some tests were made and are reported where one aspect of the modification was examined, while some other step in the procedure was not. The sequence finally settled upon in the modified procedure was not necessarily the same as for other tests reported in the following section of this thesis.

By adding an initial ashing step before the acid digestion the odor problem could be avoided. This would also allow the use of the more convenient crude fiber digestion apparatus[1], for boiling the samples and elimination of the problem of uneven boiling on the hot plate as well as the need for the steam condensors. In addition, the initial ashing would eliminate the organic matter from the sample and prevent the formation of a substance known as humin. Humin was the suspected black residue which was observed to remain adhered to the boiling flask after acid digestion, particularly with the higher normality acids. Humin is a black acid-insoluble solid formed during acid hydrolysis of protein, probably by condensation of the indole nucleus of tryptophan with small amounts of aldehydes produced during the hydrolysis (Haurowitz, 1963).

Test 7. Ashing Prior to Acid Treatment

In a series of preliminary tests samples were ashed (overnight at 650 C) prior to acid digestion and ashed again, overnight at 650 C after acid treatment and washing to remove the filterpaper (see Figure 1 for summary of

[1] Labconco Corp., Kansas City, MO.

sequences). When this procedure was applied in the AIA analysis the odor problem during the acid digestion was avoided.

The pre-ashing modification was employed in all tests reported in this section (III. C.), unless stated otherwise. To avoid the necessity for frequent descriptions of these ashing temperatures, they are presented as follows: (X-Y C) where X referred to first ashing temperature and Y referred to second ashing temperature, both temperatures expressed in degrees of Celsius. For example 650-650 C means that samples were ashed at 650 C prior to acid digestion followed by a second ashing to remove the filter paper.

Test 8. Acid Strength

Triplicate brome grass samples were analyzed for AIA content using the 4N HCl procedure as outlined (Appendix 2) but with an overnight ashing prior to acid digestion and with experimental acid strengths of 0, 0.5, 1, 2, 4 and 6N HCl. Ashing temperatures were 650-650 C. The 6N HCl was included to observe its influence on AIA compared to other normalities despite its rejection on the basis of the observations made earlier.

The lowest AIA value obtained for the sample using 1N HCl was not significantly different from 0.5N HCl which in turn was not significantly different from 2N HCl (Table 7). With 6N HCl some of the AIA may have been lost (see Test 1.), however, its mean AIA value was higher than when 4N HCl was

Table 7. Mean (±SE) AIA values of brome grass using different normalities of HCl acid.

Normality of HCl	AIA value (%)[1]	SE
0	3.840^{d}	0.064
0.5	2.854^{ab}	0.012
1.0	2.789^{a}	0.012
2.0	2.906^{b}	0.032
4.0	2.990^{c}	0.012
6.0	3.036^{c}	0.026

[1] SE of means 0.026%.

a-b Means with different superscripts are significantly ($P<0.05$) different.

used. An increased AIA value occured with increased normality from 1N to 6N HCl. This observation was consistant with those made earlier (Test 1, 5 and 6). Samples analyzed using water in place of acid, gave significantly ($P<0.05$) higher values.

Test 9. Acid Volume

The effect of acid volume (or acid to sample ratio) on AIA value for brome grass was tested using duplicate 10 g samples. Ashing temperatures were 650-650 C and acid volumes of 25, 50, 100, 200 and 400 ml of 4N HCl were used instead of the standard 100 ml.

An analysis of variance of the results of acid volume used showed a significant ($P<0.001$) difference in AIA value (Table 8). Use of acid volumes of 200 and 400 ml were not significantly different but produced values significantly lower ($P<0.05$) than when 25, 50 and 100 ml were used.

Table 8. Mean (±SE) AIA values of brome grass using different volumes of 4N HCl acid.

Volume of acid (ml)	Volume of acid per gram of sample	AIA value (%)[1]	SE
25	2.5	3.264[a]	0.017
50	5.0	3.280[a]	0.007
100	10.0	3.224[a]	0.010
200	20.0	3.132[b]	0.002
400	40.0	3.088[b]	0.027

[1] SE of means 0.016%.

[a-b] Means with different superscripts are significantly ($P<0.05$) different.

The greater volumes of acid not only increased the total amount of HCl required for each sample, but also increased the time required for samples to reach boiling point.

Test 10. Acid Temperature

This test was designed to determine the effect of acid temperature, during the acid digestion step, on estimated AIA content of brome grass. Three sub-boiling temperatures (20, 55 and 95 C) were used plus an intermittent boiling treatment. Three further boiling temperatures were used. These were based on temperature settings (low, medium and high) of the crude fiber digestion apparatus and thus influence the vigor of boiling. Observations were made in duplicate samples using ashing temperatures of 650-650 C.

Results of this experiment showed that AIA values were not significantly influenced by acid temperature at or near

Table 9. Mean (±SE) AIA values of brome grass using different acid temperatures.

Acid temperature	AIA value (%)[1]	SE
Subboiling 20 C	3.243^{c}	0.044
Subboiling 50 C	3.044^{b}	0.082
Subboiling 95 C	2.911^{a}	0.012
Intermittent boil	2.941^{ab}	0.010
Low[2]	2.909^{a}	0.002
Medium[2]	2.928^{ab}	0.003
High[2]	2.914^{a}	0.017

[1] SE of means 0.036%.

[a-b] Means with different superscripts are significantly ($P<0.05$) different.

[2] Temperature settings on the crude fiber digestion apparatus.

boiling (Table 9). Therefore, providing boiling is achieved, any temperature setting on the crude fiber digestion apparatus could be used.

Test 11. Boiling Time

The effect of acid boiling time on AIA value of samples of brome grass was examined to determine if the originally suggested boiling time of 30 min was critical for the modified procedure. A shorter boiling time would be an advantage and would allow more samples to be processed daily. On the crude fiber digestion apparatus available in our laboratory, only 12 samples could be boiled concurrently. Ashing temperatures were 650-650 C. Boiling was commenced at different times so that samples were washed free of acid

Table 10. Mean (±SE) AIA values of brome grass using different boiling times.

Boiling time (min)	AIA value (%)[1]	SE
5	3.215	0.006
15	3.218	0.003
30	3.206	0.013

[1] SE of means 0.004%.
Treatment means not significantly different.

and ashed, to remove the filter paper, at the same time.

The AIA value for the 5, 15 and 30 min. boiling times were not significantly different (Table 10). It was, therefore, concluded that boiling times of either 5, 15 or 30 min could be used to determine the AIA content.

Test 12. Washwater Temperature

Because, of the volumes of washwater used and the difficulty in maintaining a precise temperature, it was desirable to know of variations in washwater temperatures had an effect on the AIA value of samples subjected to initial ashing.

Triplicate feed samples were analyzed using washwater temperatures of approximately 20, 70, 95 and 100 C (boiling). Ashing temperatures were 650-650 C and 1N HCl was used.

The results for Test 12 are shown in Table 11. An analysis of variance of the results showed no significant difference in AIA values when different washwater temperatures were used. However, it was observed that washwater

Table 11. Mean (±SE) AIA values of brome grass using different washwater temperatures.

Washwater temperature (C)	AIA value (%)[1]	SE
20	3.246	0.016
70	3.220	0.016
95	3.204	0.023
100 (Boiling)	3.202	0.025

[1] SE of means 0.010%.
Treatment means were not significantly different.

temperatures of 95 C or higher reduced filtration time. With washwater at room temperature (20 C) the filtration time was about 20 min but at temperatures above 95 C the times were reduced to about 10 min. Therefore, for analysis washwater temperatures became important only in terms of laboratory time requirement.

Test 13. Furnace Differences

Electric furnaces[1] were used for ashing. There was possible variation in temperature and inside air circulation in and between the furnaces which would influence the AIA analysis. To determine possible effects of different furnaces on the AIA values, brome grass samples (six replications) were analyzed using four furnaces. Samples were ashed both times in the same furnace at temperatures of 650-650 C. From furnace No. 4 two samples were lost.

[1] Lindberg Hevi-Duty Box Furnace with 200-1200 C Range, Digital, Solid State Console. Canadian Laboratory Supplies.

Table 12. Mean (±SE) AIA values of brome grass obtained by using four different furnaces.

Furnace	AIA values (%)[1]	SE
1	3.234	0.008
2	3.236	0.009
3	3.226	0.010
4	3.226	0.002

[1] SE of means 0.002%.
Treatment means were not significantly different.

Acid strength of 2N HCl was used.

Values from the four different furnaces were not significantly different (Table 12).

Test 14. Acid Strength and Sample Size

In a preliminary test an attempt was made to reduce the weight of sample to 1.0 g using different acid volume to sample weight ratios. However, the results obtained when 1.0 g brome grass samples were used had to be rejected, because of unacceptable large variation in duplicate analyses (range 0.015 - 0.396%). Furthermore, it was shown earlier (Test 9) that the use of a higher acid to sample ratio resulted in significantly ($P<0.05$) lower AIA values. However, it was considered that, if sample size were reduced from 10 g to 5 g then smaller crucibles could be used and in turn more samples could be ashed concurrently in the available furnace space.

The effect of acid strength on estimated AIA content of

Table 13. Mean AIA values (%) of brome grass and feces determined by using different normalities of HCl and two fecal sample sizes.

Sample size (g)	AIA values (%)						SE of means
	Normalities						
	0.5	1	2	3	4	5	
10[1]	2.953^{a}	2.912^{a}	2.928^{a}	2.962^{a}	2.960^{a}	3.034^{b}	0.017
5[2]	5.839	5.860	5.846	5.836	5.888	5.888	0.009
10[2]	6.002	5.978	5.926	5.920	5.902	5.902	0.016

[1] Brome grass.
[2] Feces from sheep fed brome grass.

brome grass and feces from sheep fed brome grass was examined in two tests by using six normalities (0.5, 1, 2, 3, 4 and 5N) of HCl acid. Duplicate samples of brome grass (10 g) and feces (5 g and 10 g) were used. Ashing temperatures were 650-650 C in both tests.

The results are presented in Table 13. A separate analysis of variance of the brome grass results showed no significant difference between normalities, except for 5N HCl which resulted in a significantly ($P<0.05$) higher AIA value.

The variation among 5 g fecal samples was less than the variation among 10 g samples of either brome grass or feces. An analysis of variance of the results of acid strength and sample size of the fecal samples showed no significant difference between normalities used, but there was a significant

($P<0.05$) difference between the AIA values of the sample sizes used. Mean AIA values of 5 and 10 g samples were 5.860 and 5.942%, respectively. The results from earlier tests (Test 1 and 8), showed significant differences when different normalities were used. Brome grass samples were used in all tests where a significant effect of acid normality was found. In contrast, no significant effect was evident in the above test when fecal samples were used.

D. Second Modifications and their Evaluations

An initial ashing step was added to the laboratory procedure in a previous section (III. C.), but no attempt was made at that stage to examine the effect of ashing temperatures on AIA values. In this part of the study examinations were made in the first and second ashing temperatures and their effects on the estimated AIA content of a prepared grain concentrate mixture, brome grass and fecal samples. The grain concentrate mixture used was a swine ration and consisted of barley (56%), oats (20%) and wheat (10%) soybean meal (3.5%), meat meal (2%), rapeseed meal (3.5%) and alfalfa meal (2%) and supplemented with minerals (2%) and vitamins (1%). Feces were collected from two sheep that were fed the pelleted brome grass also used in the tests. The feces were bulked and well mixed before use in the present tests. All sample material was dried to constant weight in an air forced oven and ground finely through a Christy-Norris mill before laboratory analysis.

The individual steps of the laboratory procedure for each AIA method are presented in a diagrammatic scheme in Figure 1. Ashing temperatures used are indicated as before i.e., 650-650 C referring, respectively, to the first and second ashing temperatures of the analytical procedure. All ashings were done overnight. On the basis of previous tests it was decided to use 5 g instead of the 10 g sample (Test 14), 2N HCl instead of 4N HCl (Test 1, 5, 6 and 14) and 5 min instead of a 30 min boiling time (Test 11).

Test 15. First Ashing Temperature

It had been observed during preliminary tests that ashing a feed sample at 1000 C resulted in a hard, stone like residue which could not be dissolved in either water or concentrated HCl. The residue had a glazed appearance after its first ashing. In view of these observations it seemed necessary to examine the effect of first ashing temperatures on the estimated AIA content of feed and feces. Triplicate samples of brome grass, concentrate and feces were analyzed using first ashing temperatures of 150, 200, 300, 400, 500, 575, 650, 725 and 800 C. A second ashing temperature of 650 C was used.

Two values for concentrate samples (Table 14) were rejected because of obvious incomplete combustion during the second ashing step. The cause of the problem became evident later and is discussed in a subsequent section (III. E.).

An analysis of variance of the test results (Table 14)

showed a significant effect of first ashing temperature on AIA values from brome grass ($P<0.001$), concentrate ($P<0.01$) and feces ($P<0.001$). At the lower first ashing temperatures there was an increase in estimated AIA content and at temperatures higher than about 500 C there was also a significant ($P<0.05$) increased AIA content in the brome grass and fecal samples (Figure 2). Although significantly different, the 150 C first ashing temperature caused a smaller increase in AIA content of brome grass and feces than did high initial ashing temperatures. The use of a first ashing temperature lower than 200 C resulted in virtually no

Table 14. Mean AIA values of brome grass, concentrate and feces using different first ashing temperatures.

First ashing temperature (C)	AIA value (%)		
	Brome grass	Concentrate	Feces[1]
150	3.010[d]	0.813[b]	6.142[b]
200	2.880[c]	0.784[2]	5.988[a]
300	2.827[b]	0.706[2]	5.921[a]
400	2.682[a]	0.793[a]	5.915[a]
500	2.691[a]	0.779[a]	5.938[a]
575	2.801[b]	0.780[a]	6.220[b]
650	2.924[c]	0.783[a]	6.604[b]
725	3.005[d]	0.776[a]	6.815[b]
800	3.542[e]	0.788[a]	7.287[b]
SE of means	0.018	0.005	0.024

[1] Feces from sheep fed brome grass.

[a-b] Means with different superscripts are significantly ($P<0.05$) different.

[2] Mean of two values and not used in statistical analysis.

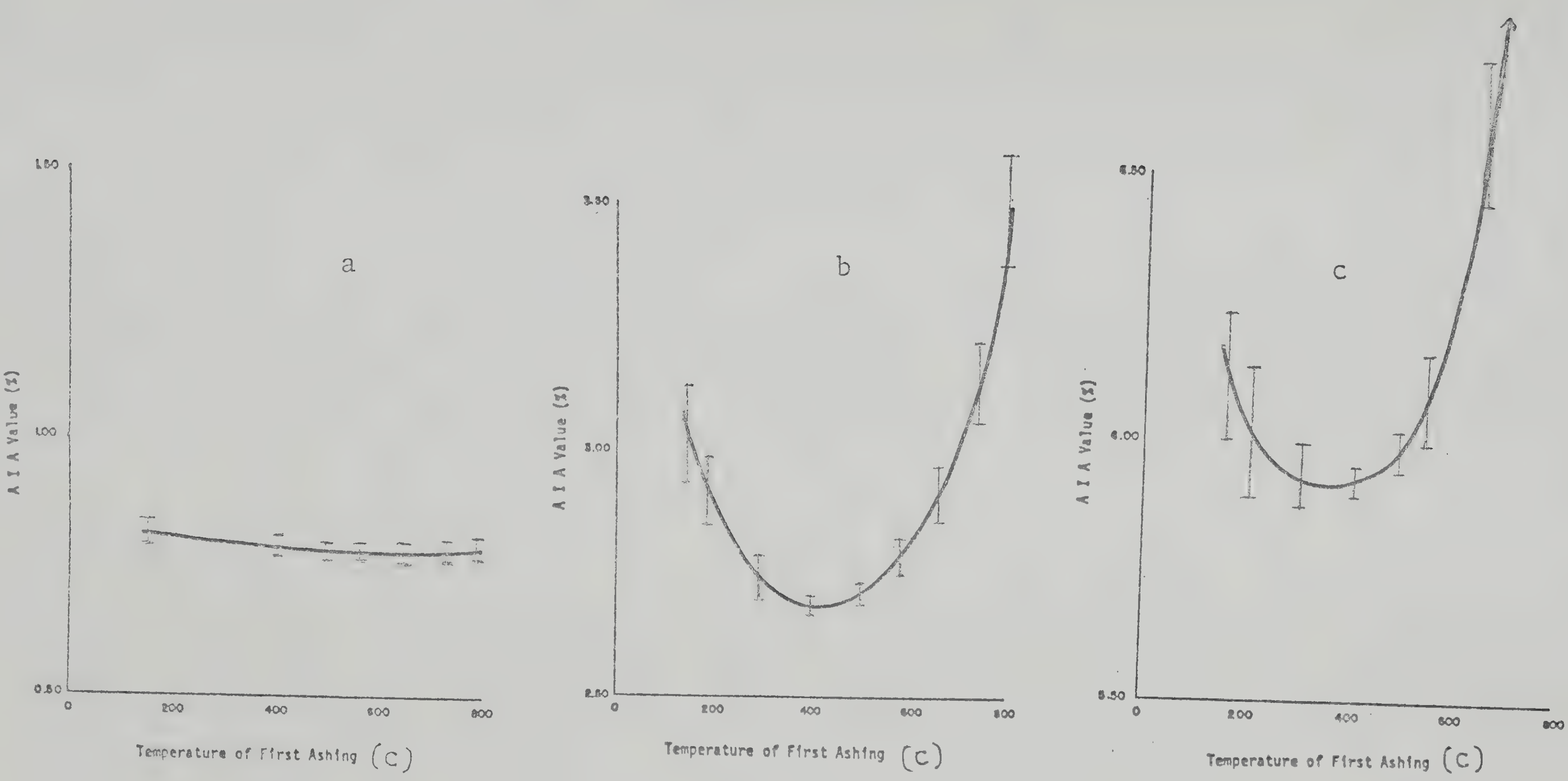


Figure 2. AIA contents of (a) swine ration, (b) brome grass and (c) feces from sheep fed brome grass, estimated by using different first ashing temperatures. Standard errors shown were multiplied by a factor of 10.

combustion of organic matter and was essentially the same as no initial ashing. On the basis of these observations in the present test it was decided that 450 C be chosen as a first ashing temperature for future tests.

Test 16. Second Ashing Temperature

Since the previous test showed a significant effect of the first ashing temperature on AIA content of feeds and feces it seemed necessary to examine the effect of the second ashing temperature on AIA values. Furthermore, if 450 C could also be used as a second ashing temperature then samples for either the first or second ashing could be done at the same time. Triplicate samples of brome grass, concentrate and feces were analyzed using second ashing temperatures of 300, 400, 450, 650 and 800 C. Samples were initially ashed at 450 C.

An analysis of variance of AIA values showed a significant ($P<0.001$) effect from the use of different second ashing temperatures for both feed and fecal samples. It is apparent, however, that a significant difference arose mainly because at the lower temperatures there was incomplete combustion of the filter paper (Table 15 and Figure 3). At temperatures of 450 C or greater there was apparently complete combustion and there were no significant differences in estimated AIA values of either feed or feces.

In view of these results it was decided that 450 C could be used as the second as well as the first ashing temperature.

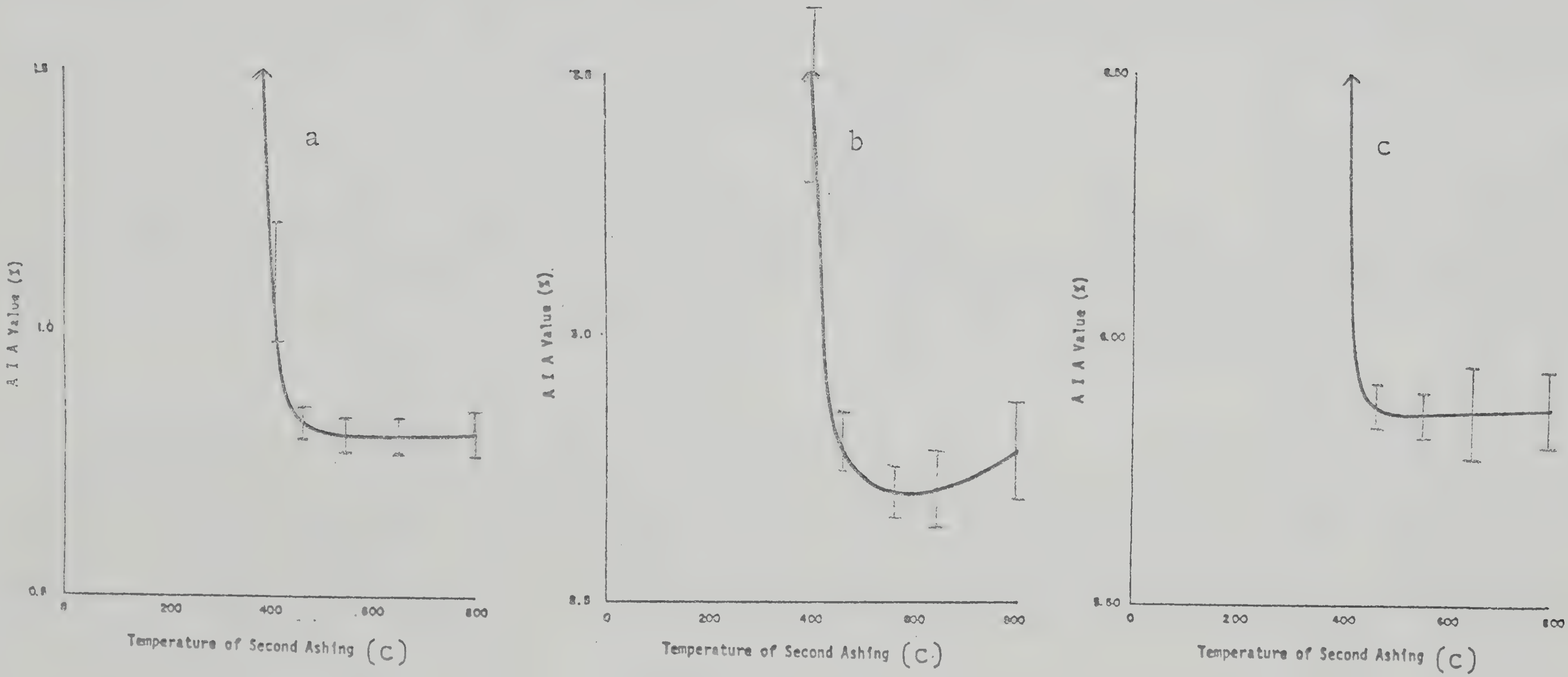


Figure 3. AIA contents of (a) swine ration, (b) brome grass and (c) feces from sheep fed brome grass estimated by using different second ashing temperatures. Standard errors shown were multiplied by a factor of 10.

Table 15. Mean AIA values for brome grass, concentrate and feces by using different second ashing temperatures.

Second ashing temperature (C)	AIA value (%)		
	Brome grass	Concentrate	Feces[1]
300	9.515^{c}	7.355^{c}	19.023^{c}
400	3.476^{b}	1.127^{b}	6.804^{b}
450	2.799^{a}	0.825^{a}	5.846^{a}
550	2.704^{a}	0.797^{a}	5.899^{a}
650	2.728^{a}	0.820^{a}	5.842^{a}
800	2.779^{a}	0.806^{a}	5.860^{a}
SE of means	0.102	0.064	0.135

[1] Feces from sheep fed brome grass.

[a-b] Means with different superscripts are significantly (P<0.05) different.

However, care must be taken that the furnace temperature stays near 450 C (±25 C) for the first ashing. The second ashing was less critical providing it remained above 400 C.

Discussion of Laboratory Procedures

On the basis of results from Test 1 to 16 the 2N HCl method was established (for detailed steps see Appendix 3 and Figure 1). The changes made to the original 4N HCl laboratory procedure that led to the 2N HCl method are discussed below. In addition observations made during the laboratory analyses, especially with regard to laboratory time and efficiency, are also discussed.

Initial Ashing

Adding an initial ashing step to the 4N HCl laboratory procedure eliminated the odor problem during acid digestion and allowed samples to be processed more quickly without the necessity of a fumehood. The initial 450 C ashing temperature also gave more consistant AIA values than no ashing or higher ashing temperatures except when a swine ration was used (Figure 2.) Furthermore, when the crude fiber digestion apparatus was used, samples could be digested in Berzelius beakers (without spout) which allowed for more even boiling of the sample mixture and easier and quicker rinsing of the beakers. The Erlemeyer flasks, suggested for use with the 4N HCl laboratory procedure, can not be used on a crude fiber digestion apparatus.

Sample and Crucible Size

Reducing the sample size from 10 to 5 g size appeared to give slightly lower AIA values probably as a consequence of the higher acid to sample ratio. The reduced sample size would facilitate obtaining sufficient sample where sample material might be limited. For example, when digesta is collected at intermediate stages along the digestive tract.

With the use of a smaller sample size it was also possible to use a smaller crucible. The 4N HCl method employed a 100 ml crucible in order to have enough space for the filter paper containing the acid digested sample. By introduction of the smaller sample size a 50 ml crucible

provided sufficient space for the initial 5 g of dried material. This not only allowed an increased number of samples to be put into the furnace at any one time, but also with the smaller crucible there was a reduced exposed surface and possible moisture absorbtion during weighing was reduced. Empty crucible weight was determined by reweighing the crucible immediately after the ash was brushed from it, see sequence of steps in analytical procedures shown in Figure 1. Predetermination of empty crucible weight, as for the 4N HCl procedure was considered to be less acurate because of possible day to day variation in the moisture content of the crucibles.

Acid Normality and Boiling Time

From the several tests on the effects of acid strength on AIA value it was not always clear that the use of 2N HCl gave lower and more consistant AIA values. However, in none of the tests was the AIA value significantly higher or more variable when 2N HCl was used rather than 4N HCl. In addition, twice as much acid is required when 4N HCl was used as compared to 2N HCl. The above with the added safety factor to laboratory staff with 2N HCl rather than 4N HCl, as far as acid vapor or direct spillage is concerned, were additional factors in changing from 4N to 2N.

In view of the nonsignificant differences with boiling times of 5 and 30 min (Test 11) and on the basis of results obtained in Test 4, it was decided to use the 5 min instead

of the 30 min boiling time as described in the original laboratory procedure. The reduction in time in this step increased by several fold the number of samples that could be analyzed each day.

Washing and Transferring the Sample

The initial ashing reduced the amount of sample remaining for acid digestion. With the small amount of ash, washwater filtered substancially more quickly than when the 4N HCl method was followed. Also, the amount of washwater required was less than half that of the 4N HCl procedure.

After washing free of acid the pre-ashed sample could be quickly and more readily transferred back into the crucible because the ash contributed minimal weight and added no adhesive properties to the filter paper. Particular care had to be taken in transferring a filter paper containing the unashed sample, to prevent breaking of the filter paper. Furthermore, if unashed samples were placed into the crucible when too wet, water at the bottom of the crucible would form steam when heated in the furnace and on occasion sample material was blown out of the crucible.

Second Ashing

The second ashing in both laboratory procedures served the same purpose, namely, to remove filter paper from the remaining ash. Results of the use of different second ashing temperatures showed that a second ashing temperature

of 450 C could be used equally as well as 650 C. By use of the same temperatures for the first and second ashing, it was possible for first or second ashings to be done in the same furnace.

E. *Furnace Capacity*

In our laboratory, limited space and electric power supply prevented the istallation of more furnaces. In order to increase the number of samples analyzed per day a perforated porcelain shelf was installed in the furnaces. This allowed 48 samples instead of the regular 24 samples (50 ml crucibles) to be ashed at one time. However, when a new furnace[1] was modified with a shelf to hold 48 samples it was found that variations in AIA values between duplicate samples were unacceptably large. It was normal procedure to repeat any duplicate analysis with a standard deviation of more than 0.050% AIA.

Despite the attainment of the prescribed temperature, it was suspected that in the new furnace there was incomplete combustion due to lack of air exchange. It was estimated that for complete combustion of 240 g of organic matter (48 samples x 5 g) approximately 20,000 l of air exchange woul be required. To test if lack of air exchange was the problem the following was undertaken. Twenty four crucibles each containing two Whatmann No 41 filter papers (about 4.7 g of paper) were placed in the furnace in known positions (Figure 4. a). A glass tube, sealed into the window of the furnace, was connected to a vacuum line via a flow meter[2]. Air was withdrawn at either 0, 1, 2, 3 and 4 l/min.

---

1. Lindberg Hevi-Duty Box Furnace with 200-1200 C Range, Digital, Solid State Console. Canadian Laboratory Supplies.
2. Roger Gilmont flow meter, serial No E-439.

In addition, one test was done with the glass tube removed and the furnace door ajar (5mm). Air was withdrawn rather than blown into the furnace to reduce the possibility of ash being blown out of the crucibles. Ashing temperatures and times were 450 C and three hours, respectively.

If complete combustion had taken place negligeble amounts of ash (less than 0.01% of the paper weights or 0.0005 g) should have remained in each crucible. Closure of the furnace door during combustion without withdrawal of air resulted in amounts of unburned material (10 to 15%) at all sites. A definite improvement in combustion was observed with active air withdrawn (Figure 4). At 4 l/min active airflow there was virtually complete combustion of the filter papers in the center of the furnace, leaving only the front and back rows with some unburned material (up to 0.05 g). In an attempt to obtain practical means for implementation during routine laboratory use of the new furnace, the door was left ajar, approximately 5 mm at the top. It appeared, however, that the partially opened door let in too much air, thereby preventing the temperature from rising sufficiently for complete and even combustion (see front three rows, Figure 4. f).

In checking out similar furnaces but ones that had been in use for several years it was observed that the furnace door contained a thin coat of black residue which prevented the door from being totally closed. The opening around the door and gaps in the fire wall apparently allowed sufficient

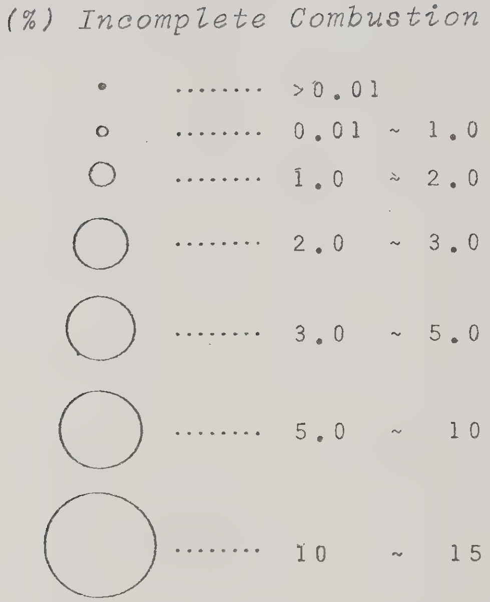
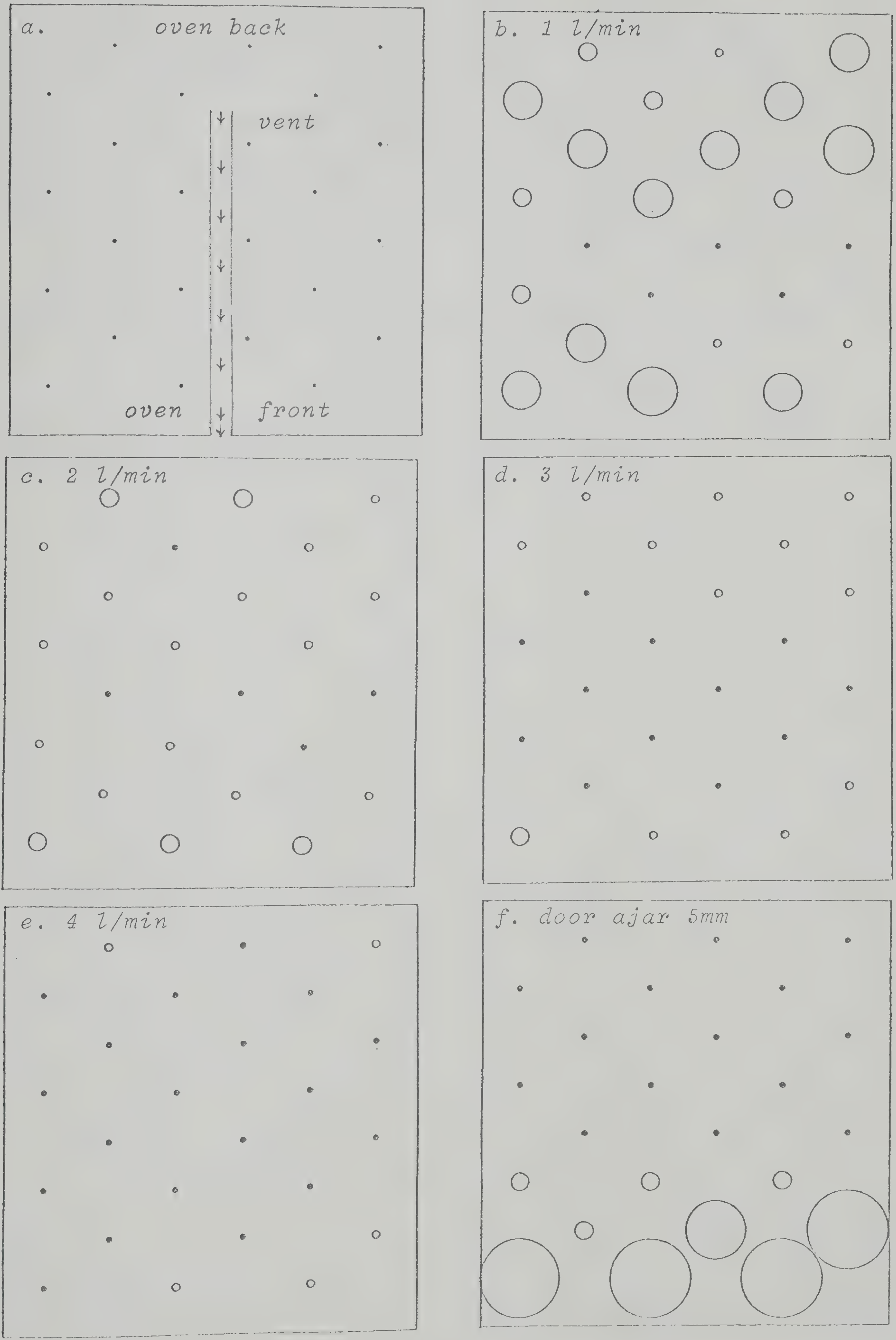


Figure 4. Schematic representation of incomplete combustion of cellulose in new furnace at 450 C.

a. Site of crucible placement and venting for oven.
b. Venting rate 1 *l*/min.
c. Venting rate 2 *l*/min.
d. Venting rate 3 *l*/min.
e. Venting rate 4 *l*/min.
f. Door ajar 5 mm.
At zero venting rate incomplete combustion of 10 to 15% occurs at all sites.


a. oven back
vent
oven front
b. 1 l/min
c. 2 l/min
d. 3 l/min
e. 4 l/min
f. door ajar 5mm

air to enter into these older furnaces, thus allowing complete combustion. From the results obtained in the above test with the new furnace it was decided to ash only 24 samples in each furnace at one time. Since the center region appeared to give the most reliable ashing results, 12 samples were placed on the bottom of the furnace and the remaining 12 samples on the shelf, both sets were placed near the center region of the furnace. Furthermore, the door of the new furnace was left ajar to maintain a space of about 3 mm between the door and the furnace.

F. *Composition of AIA*

No published information was available on the composition of AIA. Therefore, an attempt was made to obtain the chemical composition of AIA.

The following materials were analyzed: (1) AIA obtained by 2N HCl analysis of alfalfa and a barley-oats grain mixture; (2) AIA obtained by 2N HCl analyses of feces from sheep fed alfalfa and the barley-oats grain mixture; (3) ash residue from filter paper, Whatman No 41; and (4) residue of ash washings from the 2N HCl analyses.

These samples were analyzed with an X-ray fluoresence spectrometer[1] at the Alberta Research Council. Potassium content of the samples were determined by atomic absorption[2]. In addition, one AIA sample (barley-oats grain mixture) was analyzed for crystal structure by X-ray diffraction at the Alberta Research Council. Samples containing known quatities of silica, potassium and iron were prepared and used as standards. The spectrographs of the known and unknown samples were then compared and the percentage of the three major elements estimated.

The major elements detected by X-ray fluorescence spectroscopy were silica, potassium and iron. The analysis of potassium by atomic absorption showed a mean (±SD) potassium content of 0.56 ± 0.08%.

1. Model XS-25 DSR; Nuclear Diodes, Inc.
2. Techtron PTY. Ltd. Melbourne Australia.

Comparing the spectrographs of the known and unknown samples, the mean iron content of the samples were estimated at approximately 0.005%. Other elements detected could only be estimated as being present in negligible amounts. The AIA sample analyzed by X-ray diffraction showed an amorphous silicon structure.

On the basis of these analyses AIA was judged to be more than 99% silica. The presence of potassium was accounted for by the ash residue from the filter papers. Also, the presence of iron and the trace amounts of other elements could be explained in terms of ash residue from filterpaper, rather than sample residue.

G. AIA Content of Common Feedstuffs

If the AIA method is to be used it would be desirable to have some indication of the AIA contents of common feedstuffs. To obtain this information 81 samples representing some 20 common animal feedstuffs were kindly supplied by Dr. Martin of the Provincial Soil and Feed Testing Laboratory of the Alberta Department of Agriculture. These samples, with some samples of wood shavings used for animal bedding, were analyzed for AIA content by the 2N HCl method. The additional wood shaving samples were included because when the AIA method is employed to estimate digestibility it is important to know not only the AIA in the provided ration but of all material ingested by the animal. Bedding material, such as wood shavings or straw material may be consumed by the animal. This material may contain AIA and if not accounted for would bias the estimates of digestibilty coefficients. In addition, the AIA content of a sample of rumen microorganisms (bacteria) was estimated by the 2N HCl method.

The results of the AIA analysis are summarized in Table 16. A large variation between feeds and also within feeds was observed. The coefficients of variation of some feedstuffs with low AIA values were very high. The variation between AIA values within the different feedstuffs may be the result of various factors.

(1) Homogeneity of samples. Samples were allocated names according to the predominant plant spcies present, but may

contain other plant materials. Sample composition in terms of leaves to stem ratios may vary considerably. Since AIA consists of 99% silica (III. F.) and silica content varies between plant species (Russel, 1961, p. 536) as well as between parts of a single plant (Jones et al., 1963), a considerable variation in AIA values may result between two samples given the same name by the Provincial Soil and Feed Testing Laboratory.

(2) Maturation of plants at time of sampling. Mature plants are recognized to contain more silica than young plants (Jones and Handreck, 1965b).

(3) Geographic origin of sample material. Several factors such as pH of the soil (Jones and Handreck, 1965b), nutrient supply (Fletcher and Kurtz, 1964), transpiration ratios (Okuda and Takahashi, 1964) and salt concentrations (Tullin, 1954) influence the levels of silica in plants. The samples listed in Table 16 came from different regions of the province of Alberta.

In general terms, AIA values in straw were the highest, followed by hay and grains. Legumes appeared to contain less AIA than grasses. On the other hand, AIA content in wood shavings was very low. Because the wood shavings showed a low AIA content their contribution to the indigestible component of the feedstuffs is greater than to the AIA component. Therefore, wood shavings used as bedding material and consumed by the animal in appreciable amounts during a digestibility trial, will have a significant effect

on the feedstuff digestibility.

The AIA analysis of rumen microorganisms (bacteria) revealed an AIA content of 2.56%. The sample was taken from a cow grazing a brome grass pasture of undetermined AIA content.

The AIA level of these orgnisms appears to be high relative to values in many feedstuffs (Table 16). The question of the origin of the AIA in the microorganisms is therefore, raised. Also, is the AIA level of these organisms directly related to the level of AIA in the feed? At this stage these questions remain unanswered and further investigations are necessary.

Table 16. The AIA content of common feedstuffs determined by the 2N HCl method.

Feedstuff	Sample number	Dry Matter (%)	AIA value (%)
GRAINS			
Oats	4772	90.81	1.949
	3979	91.98	1.428
	4682	89.32	1.173
Barley	4794	89.44	0.812
	4789	89.76	0.506
	3978	89.44	0.877
Wheat	3926	89.50	0.133
	4768	89.80	0.190
	4769	89.33	0.161
	4593	90.99	0.228
	4594	90.58	0.078
Fababeans	4708	87.66	0.134
	4901	90.18	0.372
	4453	89.17	0.023
	4454	89.43	0.057
STRAW			
Oats	4792	85.89	1.516
	3883	93.14	1.295
	4724	90.18	3.456
	4720	92.00	2.097
	4718	92.83	1.533
Barley	4761	92.10	1.630
	3983	92.05	1.625
	4693	89.98	4.546
	4678	92.70	1.754
Wheat	4557	93.29	5.370
	4558	93.23	6.000
	4677	93.18	2.567
	4364	93.01	2.177

Table 16 (cont.)

Feedstuff	Sample number	Dry Matter (%)	AIA value (%)
Wheat	4311	94.83	4.730
HAY			
Native Grass	5325	92.19	3.440
	5326	92.55	1.065
Brome	3502	91.94	1.192
	4671	93.12	3.555
	4551	92.72	2.880
	4928	93.15	5.614
	4868	88.20	3.872
Alfalfa	4692	86.74	0.113
	4673	90.55	0.107
	4660	90.59	0.307
Grass-legume	4787	92.65	1.683
	3982	92.82	0.800
	4729	91.78	0.765
	3950	92.68	1.470
Oat	4793	91.10	0.851
	4778	93.15	1.565
	4657	92.64	1.520
	4618	92.54	1.993
	4600	84.23	2.071
Clover (sweet)	4783	91.06	0.060
	4308	92.93	0.204
Timothy	3848	92.23	1.208
	4504	88.33	3.794
	4338	93.49	1.803
SILAGES			
Oats	4650	91.80	2.189
	5042	50.58	1.973
	4998	90.51	1.905
	4925	91.33	2.206

Table 16 (cont.)

Feedstuff	Sample number	Dry Matter (%)	AIA value (%)
Grass-legume	5065	89.78	1.073
	5068	91.62	1.128
	4869	90.20	1.243
	5309	91.75	0.840
Corn Silage	4713	90.02	3.235
	4546	90.01	1.364
	4939	91.91	1.875
	4897	91.64	1.874
	4844	92.18	1.268
SPECIALS			
Dehydrated alfalfa pellets	4788	92.07	1.192
	4777	93.29	0.489
	4613	93.98	0.195
	4519	92.42	0.758
	4521	92.46	0.437
Screenings			
(rape)	4699	81.10	2.889
(flax)	5022	92.11	0.673
	4937	92.88	7.488
Supplement-grain mixed			
(hog ration)	3936	90.84	0.299
(dairy ration)	4709	90.77	1.068
(beef ration)	4663	92.35	1.556
WOOD SHAVINGS			
Cedar		91.17	0.019
Fir		91.18	0.124
Spruce		91.74	0.326
Mixture as off Truck		90.40	0.019

H. Comparison of Three AIA Laboratory Procedures

Three AIA laboratory procedures are now available. Conc. HCl (Shrivastava and Talapatra, 1962a), 4N HCl (Vogtmann et al., 1975) and 2N HCl, see Appendix 1, 2 and 3, respectively, for description of procedures and a schematic summary of each is contained in Figure 1. The Conc. HCl method was not fully described in the original publication (Shrivastava and Talapatra, 1962a), but was interpreted and used as shown in Appendix 1.

In this section is reported an examination of laboratory time and equipment requirement for the three AIA methods. Other factors such as convenience, odor and safety are also considered.

In an attempt to obtain unbiased results for each laboratory procedure the summarized data was based on observations made over an extended period of time. During this time, seventy two samples of both feed and feces were analyzed by each of the three laboratory procedures. However, analyses were done and expressed on the basis of batches of 12 samples. This was done, because the laboratory equipment for washing the samples free of acid could accommodate 12 samples in each batch.

A major difference between the three AIA procedures is the time taken for various steps and number of ashings. Where the Conc. HCl and 2N HCl laboratory procedures started with an initial ashing after sample preparation, samples analyzed by the 4N HCl procedure were acid digested before

ashing. The first ashing step eliminated objectionable odors that are associated with acid digestion, a problem especially with fecal samples. This step also eliminated the need of a fumehood and made it possible to use a crude fiber digestion apparatus. However, two ashings added time to the procedure.

Incorporation of dry matter determination at the beginning of the laboratory procedure, as in the Conc. HCl and 2N HCl methods eliminated the need for separate analysis of dry matter. Therefore, dry matter and AIA measurements were done on the same sample. Determination of empty crucible weight after the ashes were cleaned out was considered more desirable than estimation of empty crucible weight at the start of the procedure. This minimized possible variation among empty crucible weights.

The Conc. HCl method required the use of 150 ml crucibles. The 4N HCl and 2N HCl method required 100 and 50 ml crucibles, respectively. The smaller crucible had several advantages. They allowed a greater number of samples in the available furnace space and reduced the number of desiccators required for cooling the samples. The smaller surface area of the 50 ml crucibles reduced possible moisture absorption when exposed to the air during weighing. In addition, it was more convenient to handle the smaller sized crucibles.

The switch from the use of the hot plate in the fumehood (4N HCl method) to the use of the crude fiber digestion

apparatus (2N HCl method) for acid digestion provided more even boiling of all samples. It also allowed the use of a Berzelius beaker rather than an Erlemeyer flask as a container for the acid-sample mixture, thereby easing the rinsing procedure. Furthermore, putting the sample beakers on the crude fiber digestion apparatus saved time and was easier as compared to attaching the portable condensers (4N HCl procedure) to the Erlemeyer flasks.

A summary of the estimated time requirement for the three laboratory procedures is shown in Table 17. Times were divided into (a) laboratory occupancy time (requiring presence of operator) and (b) laboratory time (not requiring presence of operator). Different time periods for weighing the initial samples in each procedure, were the result of different sample weights used between procedures and the ease of transferring the weighed sample material into their respective containers.

Boiling and evaporation (acid digestion) times (Table 17 step 7), included in the total laboratory occupancy time, represents the necessary preparations that must be made before samples could be washed free of acid, such as folding filter papers, providing hot water, etc.

Rinsing containers (Table 17 and step 8) accounted for more time in the 4N HCl procedure as compared to the other two procedures. This was due to their shape and the quantity of sample they contained (Erlemeyer flasks) and it was more difficult to achieve complete transfer of residue.

Table 17. Estimation of (a) laboratory occupancy time (requiring presence of operator) and (b) laboratory time (not requiring presence of operator) required to analyze 12 samples by the three (Conc. HCl, 4N HCl and 2N HCl) AIA laboratory procedures. Note that all steps are not required for all three methods.

Step	Description	Time required (min)					
		Conc. HCl		4N HCl		2N HCl	
		(a)	(b)	(a)	(b)	(a)	(b)
1	Weighing initial samples and transfer to crucibles or Erlemeyer flasks	20		30		15	
2	Drying at 135 C		120				120
3	Weighing dried samples	10				10	
4	Ashing	10	n[1]				n
5	Transfer ash into beakers					3	
6	Addition of HCl and/or water	18		5		5	
7	Boiling or evaporating time	5	45	5	30	5	
8	Transfer of hot hydrolysate, rinsing crucibles, flasks or beakers	10		20		5	
9	Washing samples free of acid	15	45	10	20	5	5
10	Transfer samples into crucibles	1		5		1	
11	Ashing		n		n		n
12	Weighing insoluble ash and crucibles	7		7		7	

Table 17 (Cont.)

Step	Description	Time required (min)					
		Conc. HCl		4N HCl		2N HCl	
		(a)	(b)	(a)	(b)	(a)	(b)
13	Cleaning and weighing empty crucibles	10				10	
14	Weighing samples for dry matter			10			
15	Drying at 135 C				120		
16	Weighing dried samples			10			
	Total laboratory occupancy time	106		102		66	
	Total laboratory time		210+2n		170+n		125+2n
	Overall time requirement	316+2n		272+n		191+2n	

---

[1] n = overnight ashing, except for first ashing for Conc. HCl where a 5-hr ashing is required, see text.

Time required to wash samples free of acid (Table 17 step 9) was greatly reduced by an initial ashing of the samples. Ashing reduced the total amount of sample material and allowed washwater to filter through rapidly. The Conc. HCl procedure required concentrated HCl acid which increased the total washing time.

Materials and equipment required for each of the three laboratory procedures are shown in Table 18. For the greater part common available laboratory equipment was used, with the possible exception of the portable condensors (4N HCl method) for acid digestion. However, if no crude fiber digestion apparatus is available, a hot plate can be used to boil the samples. During the latter part of the studies the conventional electric kettles, used to provide hot washwater, were replaced by a continuous hot water warmer. A round bottom glass bowl (12 *l*) was connected to the distilled water source. The water in the bowl was continually heated electrically or by the steam supplied to the laboratory. In addition, a special hot water dispencer was devised to wash four samples at one time. This set-up provided a steady supply of hot distilled water and reduced total time required for washing samples free of acid.

Table 18. General observations and a comparison between the Conc. HCl, 4N HCl and 2N HCl laboratory procedures on the basis of materials (per sample) required.

	AIA laboratory procedures		
Description	Conc. HCl	4N HCl	2N HCl
No of days for analysis[1]	3	2	3
No of ashings	2	1	2
Ashing temperatures (C)	650-650	650	450-450
Odor during acid digestion	none	yes	none
Normality of HCl acid	11-12N	4N	2N
Concentrated HCl (ml)	35	32	16
Hot distilled water (ml)	300	750	300
Drying oven	yes	yes	yes
Furnace	yes	yes	yes
Steam bath	yes		
Crude fiber digestion apparatus			yes[2]
Fumehood	yes	yes	
Hot plate		yes	
Portable condensor		yes	

---

[1] Expired time from start to end of analysis. For breakdown of time requirements see Table 17.

[2] A hot plate and portable condensor can be used.

I. Comparison of AIA Methods Using Animal Feeding Trials

Experiment 1. Evaluation of AIA as a Natural Index Substances in Ruminant Digestibility Studies

The objective of this experiment was to compare the digestibility coefficients of several ruminant rations determined by the total collection method, with the digestibility coefficients estimated using the AIA natural index substance. Three AIA methods Conc. HCl, 4N HCl and 2N HCl were used and evaluated.

a. Experimental design, animal management and rations

The digestibility methods were evaluated using mature Suffolk wethers (79-86 kg), kept indoors and individually housed in fiberglass metabolism crates. Each ration (Table 19) was fed to two sheep twice daily at a level of approximately 1.2 times maintenance. These levels were calculated according to the equation presented by the National Research Council (NRC, 1968) which relates the mainenance metabolizeable enery requirement to metabolic size.

$$\text{Kcal} = 112 \times \text{body weight (kg)}^{0.75}$$

Feeding times were 0930 and 2100 hours. Calcium and phosphate in the form of ground limestone and dicalcium phosphate were provided to NRC requirements and put into the feed each time the animals were fed. Sand (20.0 g) added to rations 4, 5 and 6 (Table 19), was mixed with the feed and moistened to prevent settling out, before the animals were fed. Water

Table 19. Percentage composition of rations

Ingredients	International reference number	Ration 1	2	3	4	5	6	7	8
Alfalfa hay, pelleted	1-00-068		50	100		49.4	98.6		
Barley grain	4-00-549	48.7	25		48.1	24.7			
Oats grain	4-03-388	48.7	25		48.1	24.7			
Brome grass[a], pelleted	1-00-890							100	100
Sand					1.3	1.2	1.1		
Ground limestone	6-02-632	1.6			1.5				
Dicalcium phosphate		1.0			1.0				
Analyzed composition (moisture-free basis)									
Dry matter (%)		91.2	93.6	96.1	91.2	93.6	96.1	90.9	91.8
Crude protein (%)		10.4	12.4	14.4	10.4	12.4	14.4	11.3	12.6

[a] Brome grass (*Bromus* spp.) from different sources and considered as different rations.

[b] Sand (acid-boiled and washed) consisted of partical sizes: .149 mm 34.6%; 149-.420 mm 25.7%, .420-.590 mm 12.8%; .590-.840 mm 34.6%.

was available at all times and fresh water was given once daily.

The choice of ration for the present test was based on predicted differences in chemical composition, AIA content and digestibility. Alfalfa pellets used were supposably from a single batch from a commercial plant. They were obtained in paper bags of 22.7 kg weight. No attempt was made to remix the pellets after delivery. Rations 7 and 8 (Table 19) consisted largely of brome grass (*Bromus* spp.) and some crested wheatgrass. They came from two different batches and were considered as two different rations. The concentrated barley-oat grain ration was prepared in a single batch at the University of Alberta feed mill. The sand added to rations 4, 5 and 6 was obtained by ashing (overnight at 650 C) black garden soil. The ash residue that passed through the screen (0.840 mm), was washed in diluted HCl, washed free of acid and dried. Partical sizes of the remaining material, termed sand, were determined by water washing through a series of sieves.

b. Feed and fecal collections

A minimum 30-day preliminary period was allowed for the animals to adjust to each ration before the 8-day collection period. During the 8-day period daily fecal samples were collected every 24 hr (0900 hr) from metal trays lined with nylon window-screening beneath the crate floors. Total daily fecal outputs were weighed using a top loading balance

to the nearest one-tenth of a gram and dried to constant weight in aluminum pans at 55 C in a forced air oven for three days. Feed samples were collected each day and dried in the same manner as the fecal samples. Both, feed and fecal samples were finely ground using a Christy-Norris mill[1] and stored in polyethylene bags. Prior to laboratory analysis, samples representing a constant proportion of daily feed intake and daily fecal output of individual sheep were combined to make one composite sample of the 8-day collection period. Composite feed samples did not contain sand, ground limestone or dicalcium phosphate. The sand, ground limestone and dicalcium phosphate were analyzed independently for AIA content.

c. Analysis and calculations

The dry matter contents of feed and fecal samples were determined as prescribed by the AOAC (1975). Nitrogen was determined by the macro-Kjeldahl method (AOAC, 1975).

AIA contents of feed and fecal samples were calculated with equations as shown (Appendix 1, 2 and 3) for each AIA method.

Apparent dry matter (DM) digestibility coefficients were calculated with the formula

$$\%\ \text{Apparent DM digestibility} = 100 \times \frac{\text{feed DM (g)} - \text{fecal DM (g)}}{\text{feed DM (g)}}$$

---

1. Model No. 8, Chelmsford, England.

d. Statistical analysis

Analysis of variance and regression analysis were done with computer programs available from the University of Alberta Computing Centre. Duncan's New Multiple Range Test (Steel and Torrie, 1960) was used in comparison of means.

e. Results

The AIA content (Table 20) of the rations ranged from 0.15 to 3.08%; the lowest values were found in alfalfa (ration 3) and the highest in brome grass (rations 7 and 8). Addition of sand increased the AIA content of the rations by 1.45 to 1.80%. There were differences ($P<0.05$) in the estimation of AIA obtained by the three AIA methods further indicating the empirical nature of the AIA method and the importance of consistancy of laboratory analysis. Repeatability of duplicate analysis was 0.02% AIA or better for all three methods and duplicate analyses which differed by more than this amount were repeated.
Approximately 8% of all analyses needed to be repeated.

A linear, significant ($P<0.01$) and positive relationship was found between digestibility coefficients from the three AIA methods (Conc. HCl, 4N HCl and 2N HCl) and the total collection method (Table 21 and Figure 5). There was no significant difference ($P<0.05$) in variance and slope between the three individual regressions. Intercepts of the regressions of total collection and Conc. HCl and 2N HCl were not significantly ($P<0.05$) different but the intercepts

Table 20. Percentage AIA in rations determined by three AIA methods.

AIA method	Rations[1]								Mean
	1	2	3	4	5	6	7	8	
Conc. HCl	1.42	0.81	0.21	3.06	2.60	2.01	3.08	3.05	2.03^a
4N HCl	1.23	0.68	0.16	2.70	2.29	1.91	2.85	2.82	1.83^b
2N HCl	1.40	0.70	0.15	2.85	2.36	1.90	2.93	2.87	1.90^c

[1] See Table 19 for ration composition.

$^{a-c}$Means with different superscripts are significantly ($P<0.05$) different. (SE of means 0.021%).

Table 21. Relationships between dry matter digestibility coefficients (%) determined by the total collection (TC), 4N HCl and 2N HCl methods.

Regression	r	SE^b	RSD
TC = 6.72 + 0.87 Conc. HCl	0.95**	±0.11	±2.74
TC = 7.23 + 0.90 4N HCl	0.96**	±0.11	±2.66
TC = 4.86 + 0.89 2N HCl	0.98**	±0.08	±2.01

r= Correlation coefficient.
SE^b = Standard deviation of the regression coefficient.
RSD = Standard error of estimation.
** ($P<0.05$)

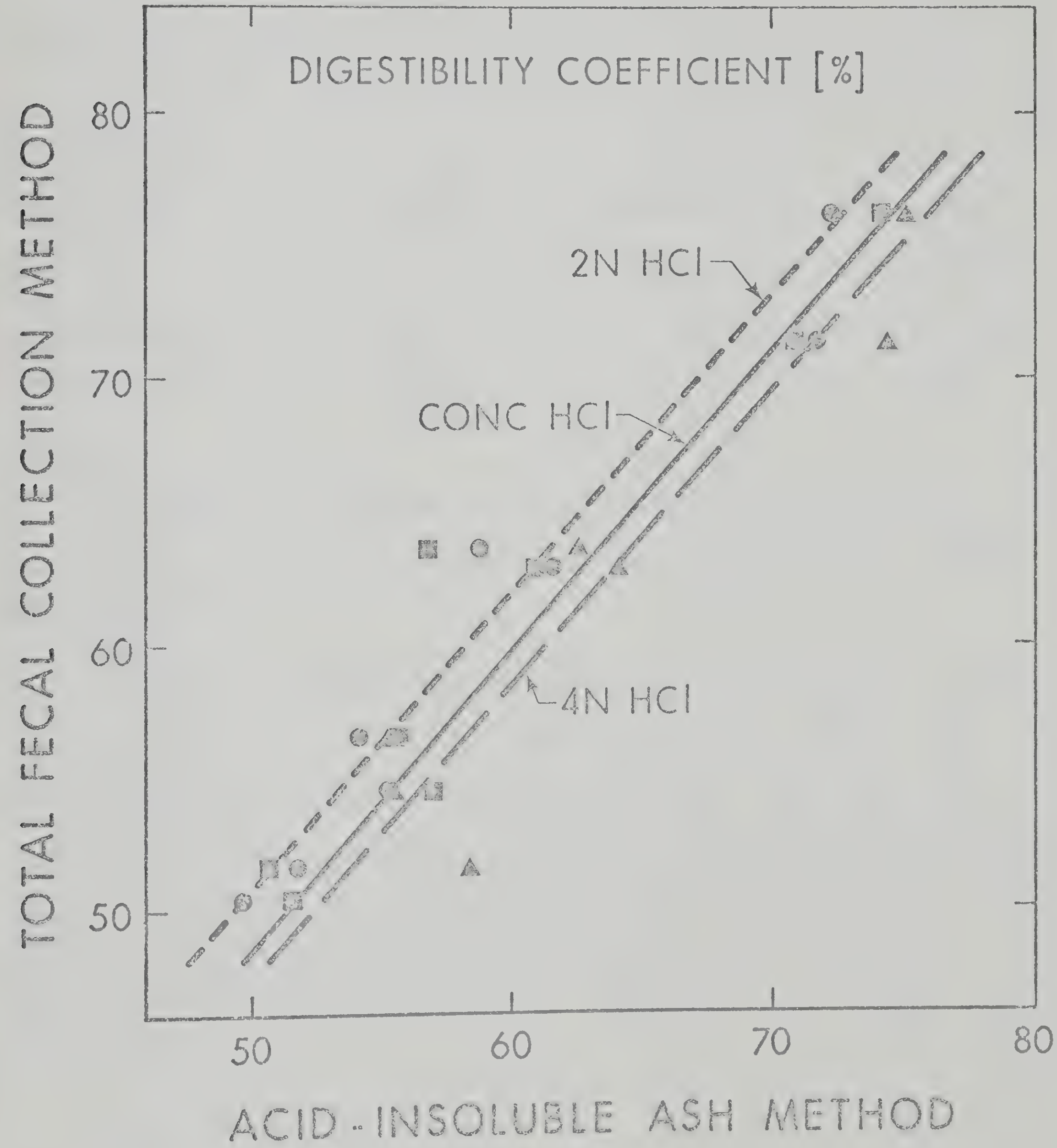


Figure 5. Relationships between dry matter digestibility coefficients from three AIA methods (Conc. HCl, 4N HCl and 2N HCl) and the total collection method.

for both these regressions were different ($P<0.05$) from the regression involving the 4N HCl method.

An analysis of variance of the digestibility coefficients showed that most of the variance arose from differences in rations (Table 22) but there was also a significant ($P<0.05$) difference between various AIA methods (Table 23). While none of the AIA methods differed significantly from the total collection estimates of digestibility, the 4N HCl method provided estimates which were significantly ($P<0.05$) greater than those estimated by the Conc. HCl and 2N HCl methods.

The mean digestibility coefficients of comparative rations with or without sand were not significantly different (Table 23).

Using the measurements of feed intake and fecal output and AIA content in each, estimates were obtained of the percent AIA recovery in feces (Table 24). The mean AIA recovery using the 4N HCl method was significantly ($P<0.05$) greater than for the other two methods. However, none of the means differed significantly from total (100%) AIA recovery.

Table 22. Analysis of variance of the effects of rations and AIA methods on estimation of dry matter digestibility coefficients (%).

Source of error	df	Mean Square	F-value
Rations	7	303.98	103.04**
Methods	3	11.77	3.99*
Error	21	2.95	
Total	23		

df = degrees of freedom.
*P<0.05
**P<0.01

Table 23. Mean dry matter digestibility coefficients (%) of rations (Table 19) estimated by total collection and three AIA methods. Two animals were used for each ration.

Ration	Total collection method	AIA method			
		Conc. HCl	4N HCl	2N HCl	Means
1	76.2	74.1	75.0	72.2	74.4^{a}
2	63.0	61.0	64.1	61.5	62.4^{b}
3	51.7	50.8	58.2	51.7	53.1^{cd}
4	71.5	70.9	74.4	71.7	72.1^{a}
5	63.7	56.7	62.5	58.7	60.4^{b}
6	50.3	51.6	51.6	49.7	50.8^{d}
7	56.5	55.6	55.1	54.1	55.3^{c}
8	54.4	57.0	55.4	55.2	55.5^{c}
Mean	60.9^{ab}	59.7^{a}	62.0^{b}	59.4^{a}	

$^{a-d}$Means with different superscripts are significantly (P<0.05) different. (SE of means 0.61%).

Table 24. Mean recovery (%) in feces of AIA. Two sheep were used for each ration. AIA content in feed and feces was determined by three AIA methods.

Ration	Conc. HCl	4N HCl	2N HCl	Means
1	91.8	95.2	85.6	90.8^{d}
2	95.1	103.3	96.4	98.3^{bc}
3	98.3	115.6	100.7	104.9^{a}
4	97.5	110.9	100.3	102.9^{a}
5	84.0	96.9	87.8	89.6^{d}
6	102.6	102.6	98.8	101.3^{ab}
7	98.1	97.0	95.0	96.7^{c}
8	106.1	102.3	101.9	103.4^{a}
Mean	96.7^{a}	103.0^{b}	95.8^{a}	

[a-d] Means with different superscripts are significantly ($P<0.05$) different. (SE of means 1.44%).

f. Discussion of Experiment 1

In digestion trials with ruminants the length of the preliminary feeding periods has been dictated largely by the length of time required for the feed intake by the animals to become constant and for the feed residues of the previous ration to be eliminated. However, there is increasing evidence that the composition of a diet has marked influence on the microflora of the rumen (Warner 1962; Hungate 1957, 1966, Grubb and Dehority, 1975). Several workers have come to the conclusion that the optimum length of preliminary feeding period depends on the nature of the change in diet (Nicholson et al., 1956). When radical changes in the ratio of hay to concentrates are involved, a 16-day preliminary period may not be enough to obtain reliable digestibility values; the required time may lie between 16 and 30 days. However, when the change in rations affects only the nature of the nitrogen supplements while the basic components remained constant, a preliminary feeding period of 17 days appears to be adequate. The idea that the time required for adaptation should vary with the nature of the change in diet seems reasonable, since it would depend on the nature and the magnitude of the differences in microbial population in the rumen of animals conditioned to the two rations. In the present study feed changes were made from a complete alfalfa to a complete concentrate ration. Therefore, a minimum 30-day preliminary period was chosen to allow the animals to fully adjust

to each ration.

A highly significant (P<0.01) effect of rations on DM digestibility (Table 22) was not surprising, because the rations chosen for this experiment varied in chemical composition. The estimated digestibilities of the different rations were affected by the AIA methods (Table 22), but a significant interaction between AIA methods and rations was not present.

The standard errors of estimation of the mean digestibility coefficients determined by the three AIA methods were not significantly different. This is an indication that the variability of the digestibility coefficients of the three AIA methods were of the same magnitude.

The greatest difficulty in assessing a digestibility trial lies in the fact that no exact standard is available. Accepting either the traditional total collection method, which is the normal procedure in digestibility studies or the ratio method such as the AIA method, implies accepting a number of variations. With the total collection method DM intake and DM output are based on weighing all of the material consumed and excreted by the animal. Weighing in itself is critical, because weighing errors lead to biased digestibility estimates and once a weighing error is made it can not be corrected at a later date. Selective feeding by the experimental animal (non-pelleted ration) demands a decision from the experimentor to verify if the weighback

represents a uniform mix of the feed offered. Simple subtraction leads to a biased digestibility estimate. Furthermore, losses do occur. Feed is lost in either feces, urine or simply on the floor. When large experimental animals are used in digestibility trials the losses of feed and feces could be substantial.

Collecting all fecal material is in a practical sense difficult to accomplish. When fecal material is soft any surface coming into contact with such feces removes part of the total amount. Petry and Enders (1974) found that the dirtiest pigs had the lowest fecal recovery. Furthermore, when soft feces are contaminated with feed, separation becomes impossible without losing some of the fecal material. Contamination of feces with urine is also difficult to prevent.

Experiment 2. Comparison Between the 2N HCl and Total Collection Methods in Estimating Digestibility by Sheep

The experiment reported in this section was designed to evaluate the AIA natural index substance method (2N HCl) as a method for estimating digestibility of ruminant feedstuffs compared to digestibility estimated by the total collection method. Three experimental rations which differed in AIA content and chemical composition were used in the study.

a. Experimental design, animal management and rations

The experimental design consisted of a modified 3 x 3 latin square with 3 periods (each period incorporated an adaptation phase of 30 days and a collection phase of 8 days), 3 ration and 3 sheep units (each unit consisting of two sheep). Two methods for determining digestibility coefficients (total collection and 2N HCl) were imposed on each of the above treatment combinations. Rations 1, 2 and 3 of previous experiment (Table 19) were used.

Six Suffolk wethers (74 to 86 kg) about two years of age were used for the experiment. All animals were in good physical condition.

The sheep were allotted the three units of two wethers per unit on the basis of body weight, so that animals among treatments were as uniform as possible. The animals were kept indoors in individual metabolism crates.

Two sheep (unit) received each of the three rations

used (Table 19) at one time. Preliminary period, collection period, level of feeding and feeding times were the same as described in the previous experiment (Experiment 1). The rations were calculated on the basis of animal weight at the start of the experiment and held constant thereafter regardless of weight changes.

Feed and fecal collections, calculations and statistical analysis were the same as described in previous experiment (Experiment 1).

b. Analysis

Triplicate gross energy determinations of feed and feces were determined by a Parr oxygen bomb calorimeter[1]. Further analysis were done as described in previous experiment (Experiment 1).

c. Results

Dry matter digestibility coefficents and dry matter digestible energy (DMDE) coefficients of three rations, estimated by the total collection and 2N HCl methods are shown in Table 25. From these results it is obvious that the digestibility values obtained by the 2N HCl method for ration 3 (period 2) were unrealistic. Also, the digestibility values obtained for ration 3 (period 3) by the same method showed a large deviation (8-10%) from digestibility values obtained by the total collection method.

1. Model No. 101 A, Parr Instrument Corp., Moline. ILL.

Table 25. Mean percent digestibility of dry matter and of energy on dry matter basis for three rations estimated by the total collection (TC) and 2N HCl methods.

Period	Ration	Animal	Animal unit	DMD[1]		DMDE[2]	
				TC	2N HCl	TC	2N HCl
1	1	74	1	76.9	73.9	78.6	75.6
		83		75.2	71.0	76.4	72.2
	2	51	2	62.1	65.4	61.2	64.6
		49		63.4	67.6	62.8	67.1
	3	50	3	50.3	51.8	46.6	48.2
		77		52.4	46.7	52.8	47.1
2	1	50	3	77.7	74.5	80.2	77.3
		77		75.3	72.7	76.3	73.8
	2	74	1	67.5	63.9	67.5	63.9
		83		64.3	60.2	64.2	60.0
	3	51	2	54.4	-28.0	55.2	-25.7[3]
		49		54.1	10.7	52.3	7.1[3]
3	1	51	2	76.3	73.8	78.4	76.3
		49		70.9	73.0	73.5	75.4
	2	50	3	58.2	59.0	57.8	58.6
		77		58.1	67.1	57.9	66.9
	3	74	1	50.8	61.1	50.6	61.0
		83		49.7	58.1	49.0	57.6
Mean				63.2	63.8	63.3	64.0
SE of mean				0.70	0.74	0.74	0.78

---

[1] Dry matter digestibility.

[2] Dry matter digestible energy.

[3] Coefficients not included in the treatment mean.

Daily feed samples (ration 3) during the 8-day collection period, of each period were analyzed for AIA content (Table 26). The variation in AIA content among daily alfalfa samples remained relatively constant in period 1, but incresed sharply at the end of period 2. This was the reverse showing of period 3 where AIA values were high initially but dropped sharply at the end of the 8-day collection period. On the basis of these observed daily variations of AIA values for alfalfa, it was decided to reject ration 3 (period 1, 2 and 3) in this experiment. Ration 3 (period 1) was rejected, despite relatively constant AIA values, because its validity became questionable. When rejecting ration 3 then ration 2, which consisted of 50% alfalfa (ration 3), also had to be rejected.

The mean digestibility coefficients for ration 1, estimated by the total collection and 2N HCl methods, were 75.4%[1] and 73.2%[1], respectively, and these coefficients were not significantly different. Mean digestible energy coefficients for ration 1 estimated by the total collection and 2N HCl methods were 77.2%[2] and 75.1%[2], respectively, and were not significantly different.

Mean (±SE) AIA recovery in feces from sheep fed ration 1 during the three 8-day fecal collection periods was 91.8 ± 3.76%.

[1] SE of means 0.56%.

[2] SE of means 0.49%.

Table 26. Percent AIA of alfalfa (ration 3) on individual days during three 8-day fecal collection periods.

Period	Ration	Day							
		1	2	3	4	5	6	7	8
1	3	0.228	0.245	0.245	0.232	0.192	0.201	0.208	0.197
2	3	0.191	0.168	0.156	0.190	0.178	0.172	0.340	0.799
3	3	1.158	0.866	0.878	0.931	0.850	0.916	0.183	0.165

d. Discussion of Experiment 2

The importance of a constant AIA level in the feed, when using the AIA index substance to determine digestibility, became obvious in this experiment. During this experiment the sheep were fed alfalfa (ration 3) from the commercial prepared bags each of approximately 22.7 kg. The bags were opened in random order and each was sufficient for approximately four days. Daily feed samples taken (approximately 400 g) for composited samples, allowed AIA analysis on a daily basis (Table 26) and also indicated the large variation from bag to bag.

Analysis of the samples after completion of the feeding phase of the experiment clearly showed that the alfalfa feed was of two distinct types, one with an AIA content of 0.15 to 0.24% and the other containing 0.80 to 1.16%. Unfortunately the problem was not discovered untill too late and thus the necessity to reject not only ration 3, but also ration 2 since it consisted for 50% of ration 3.

AIA values of the concentrate (ration 1) ration on individual days during the different collection periods did not show the above variation. For example, the lowest and highest AIA contents of ration 1 during the 8-day collection period of period 2 varied by approximately 7%, whereas ration 2 remained relatively constant during the first six but increased sharply in AIA content during the last two days (20% the seventh and 60% the eighth day).

It is evident (Table 26) that random sampling of alfalfa

could not have been used in this experiment to determine digestibility by the AIA method. Since the AIA content of alfalfa was low to start with, variations in AIA values would lead to a greater variation in digestibility.

J. Experiment 3. Diurnal Variation

To obtain an accurate estimate of feedstuff digestibility by any of the index substance methods it is essential to obtain representative samples of feed and feces. A diurnal variation in the excretion of an index substance in feces would create a problem unless the excretion pattern was established and the sampling of feces done to adjust for the variation. Tests were, therefore, conducted to determine if there was a diurnal excretion pattern of fecal AIA.

1. Experimental

A series of 2-hourly fecal collections were made in conjunction with Experiment 2. Collections were started immediately following the 8-day collection periods of Experiment 2. Thus the animals had a minimum of 38 days to adjust to their rations.

Animal management was indentical to that described in the previous experiment (Experiment 2). Incandesant lighting illuminated the Metabolic Unit during the day and was turned off during the night except for short periods when samples were collected.

Feed and fecal samples were weighed, dried in a forced air oven at 55 C to constant weight and reweighed. Each sample was finely ground using a Micro Analytical Mill (Canadian Laboratory Supplies) and stored in a polyethylene bag.

AIA content of feces were determined by the 2N HCl method (Appendix 2).

2. *Results and discussion*

Samples collected from sheep fed rations 2 and 3 were rejected because of possible variation in the AIA content of the feed used immediately prior to the collections. This problem is described in the previous experiment. Only collections from sheep recieving ration 1 (barley-oats grain) were utilized.

Shown in Table 27 are the AIA values of the 2-hourly collected fecal samples of each animal. the "missing" values were intervals of day during which the sheep did not defecate. An analysis of variance of the effect of time on the AIA content of feces did not show a significant time effect. However, there was a significant (P<0.001) difference between the mean AIA values of the six animals. Animal 49 had a much lower AIA content in its feces during the 24-hr period, the reason for this is not known.

To normalize the values and remove animal effects, each 2-hr AIA value for each animal was expressed relative to the mean value for the respective sheep (Table 28). However, an analysis of variance did not show any significant time effect.

From the above results it was concluded that there was no significant variation in diurnal excretion of AIA and that for digestibility studies a random fecal sample could

Table 27. The AIA values of fecal samples collected at 2-hourly intervals during a 24 hour period and estimated by the 2N HCl method.

Time of day	AIA values(%)					
	Animals					
	74	83	50	77	51	49
0600		5.53		5.11		
0800	5.34		5.43	5.05	5.13	
1000	5.42	5.30	5.54	5.51	5.49	4.58
1200		5.24	5.51	5.58		4.41
1400	5.37	5.39	5.45	5.48	5.49	4.32
1600	5.26	5.48		5.40	5.42	4.38
1800	5.30		5.68	5.48	5.32	4.50
2000	5.23	5.49	5.62	5.34		4.54
2200	5.45	5.52	5.62	5.29		4.50
2400	5.21			5.16	5.29	
0200	5.21			5.14	5.33	4.46
0400		5.60		5.08	5.23	
Mean	5.31	5.44	5.55	5.30	5.34	4.46
S.E.	0.030	0.043	0.005	0.054	0.044	0.031

Table 28. AIA values of fecal samples collected at 2-hourly intervals during a 24-hour period, as determined by the 2N HCl method and presented as a percentage when the mean AIA value of each animal was expressed as 100.

Time of day	Animals					
	74	83	50	77	51	49
0600		102		96		
0800	101		98	95	96	
1000	102	97	100	104	103	103
1200		96	99	105		99
1400	101	99	98	103	103	97
1600	99	101		102	102	98
1800	100		102	103	100	101
2000	98	101	101	101		102
2200	103	101	101	100		101
2400	98			97	99	
0200	98			97	100	100
0400		103		96	98	

have been taken at any time of the day (Figure 6). Furthermore, recent work with horses and pigs (John McCarthy, personal communication) has similarly failed to show diurnal variation of AIA in feces of these animals.

One animal (49) showed consistently lower AIA values during the 24-hr period, reflected in the lower digestibility values (Figure 6). No explanation was found for this variation.

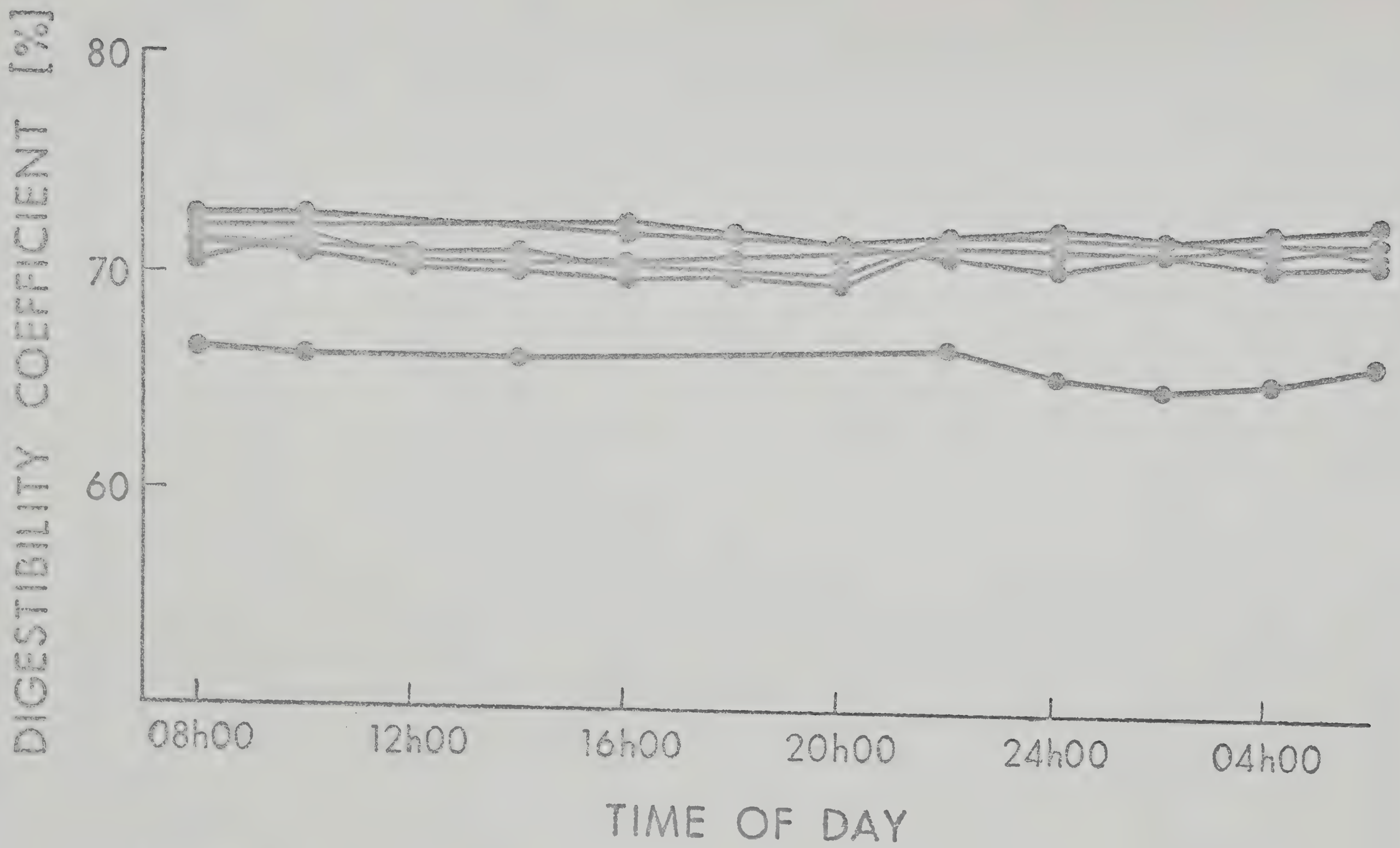


Figure 6. Dry matter digestibility coefficients of a barley-oats grain ration estimated by the 2N HCl method from samples of feces taken at 2-houfly intervals during a 24-hr period. Each line represents results from one sheep.

K. Comparison between Total Collection and AIA Estimated Coefficients of Digestibility

The acceptability of the AIA method for determination of digestibility will depend largely on favorable comparison with the traditional total collection method for determination of digestibility. The acceptance of the method by the researcher should not, however, soly depend on the outcome of these conparisons, because the AIA method possesses some distinct advantages over the total collection method. The major advantage is avoidence of the necessity for total collection of feces by determining digestibility from an aliquot of feed intake and fecal output. To obtain a further assessment between these two methods, results from a number of digestibility studies involving total collection were obtained from other reseachers and compared with results using the 2N HCl method. Feed and fecal samples collected by the researcher were analyzed for AIA by the 2N HCl method (Appendix 2) and digestibility values estimated. The samples were from pooled collections over periods of 6-8 days.

Study A.

The digestibility coefficients of a pelleted hay ration used by Westra (1975) in a study on the effect of temperature on digestion in sheep, are shown in Table 29. The hay ration consisted largely of brome grass (*Bromus* spp.) and some crested wheat grass.

There was no significant difference between the two

Table 29. AIA recovery and dry matter digestibility coefficients estimated by the total collection (Westra 1975) and the 2N HCl methods.

Sheep	Dry matter digestibility (%)			AIA recovery (%)
	Method			
	Total collection	2N HCl	Difference	
98	53.5	55.0	+1.5	103.5
92	54.4	54.5	+0.1	99.9
96	58.7	57.1	-1.6	96.2
94	53.6	56.1	+2.5	105.6
90	55.5	55.0	-0.5	98.9
97	54.9	56.8	+1.9	104.5
98	53.4	55.7	+2.3	105.0
90	53.1	53.2	+0.1	100.1
94	53.5	56.0	+2.5	105.8
Mean	54.5	55.5	+1.0	102.2
SE	0.59	0.40	0.49	1.16

methods. Total AIA recovery from the feces varied from 98.9% to 105.8% with a mean recovery of 102.2%.

On the basis of these results the 2N HCl method compared well with the total collection method in estimating digestibility from a hay ration.

Study B

Table 30 shows the digestibility coefficients estimated by the total collection and 2N HCl methods obtained from an experiment with sheep in which the effects on nutritive value of barley straw were being studied (Weisenburger, 1976).

Mean digestibility coefficients between the two methods were not significantly different. There was, however, a large variation between the estimated digestibility coefficients from sheep 67. No explanation was found for this variation.

Total AIA recovery from the feces varied from 87.2% to 119.1% with a mean AIA recovery of 101.0%.

Study C

Table 31 shows the digestibility coefficients of long alfalfa-brome hay estimated by the total collection and 2N HCl methods from an experiment in which beef cows were exposed for prolonged periods of various temperatures in a controlled (18 C) and cold outdoor (-20 to 0 C) environment (Christopherson, 1976).

Table 30. AIA recovery and dry matter digestibility coefficients estimated by the total collection (Weisenburger, 1976) and the 2N HCl methods.

Sheep	Dry matter digestibility (%)			AIA recovery (%)
	Method			
	Total Collection	2N HCl	Difference	
66	51.1	51.9	+0.8	101.5
65	49.7	50.0	+0.3	100.4
67	47.4	55.9	+8.5	119.1
70	52.1	52.9	+0.8	101.6
69	61.5	57.8	-3.7	91.1
76	66.7	61.8	-4.9	87.2
78	61.8	63.7	+1.9	105.3
79	61.3	62.2	+0.9	102.4
64	53.1	54.0	+0.9	102.0
68	54.3	55.5	+1.2	102.7
74	56.1	57.7	+1.6	103.7
75	54.6	51.9	-2.7	94.5
Mean	55.8	56.3	+0.5	101.0
SE	1.68	1.29	0.97	2.29

Table 31. AIA recovery and digestibility coefficients estimated by the total collection (Christopherson, 1976) and the 2N HCl methods.

Cow	Dry matter digestibility (%)			AIA recovery (%)
	Method			
	Total collection	2N HCl	Difference	
32	60.6	64.2	+3.6	90.8
31	60.9	58.5	-2.4	96.7
32	61.2	70.4	+9.2	76.8
31	62.0	68.6	+6.6	75.5
32	61.6	71.7	+10.1	73.8
31	62.1	66.9	+4.8	79.5
Mean	61.4	66.7	+5.3	82.2
SE	0.25	1.96	1.85	3.81

Digestibility coefficients (Table 31) as estimated by the 2N HCl method varied markedly from those estimated by the total collection method. The mean digestibility coefficients between the two methods were significantly ($P<0.05$) different. In this study the long alfalfa-brome hay ration was fed directly from the bale. There could have been a variation in the AIA content of the ration as a consequence of variation in ratio of alfalfa to brome grass in each bale. Furthermore, it is sometimes difficult to obtain representative samples of the hay contained in bales. As there is a large difference in AIA content of alfalfa and brome grass (III. G.) a small difference in the ratio of alfalfa to brome hay could result in a large difference between the AIA values of samples taken from the hay mixture. In fact the AIA values of the three feed samples saved from the experiment were 0.360, 0.266 and 0.257% with crude protein values of 14.3, 15.1 and 14.6%, respectively. While the low AIA values of these feed samples indicated that the ration consisted mostly of alfalfa hay, the variation in AIA and protein values are indicative of changes in the proportion of alfalfa and brome grass. If there were day to day changes in the proportions of alfalfa and brome grass, as different bales were used, and consequently changes in AIA values of the feed, then reliable estimates of ration digestibility by the AIA method could not be expected.

The problem encountered in this study appeared to be similar to that in Experiment 2, where alfalfa was one of

the experimental rations. In both cases, a ration with a low AIA value was involved. Therefore, when the 2N HCl method is used to estimate digestibility of a ration particular care must be taken to ensure constancy of the ration fed.

IV GERNERAL DISCUSSION

During the laboratory evaluations of the AIA methods it was established that the 2N HCl method as compared to the Conc. HCl and 4N HCl methods was more convenient and less time consuming. The addition of an initial ashing step (Figure 1) not only increased the number of samples that could be analyzed per day, but also eliminated the odor problem associated with the 4N HCl method.

In digestibility studies with sheep the three AIA methods showed no significant difference between their coefficients of digestibility for the different feeds. When compared to the results calculated by the total collection method, the coefficients of digestibility estimated by the four methods were not significantly different. These results were in agreement with those that other workers have obtained with sheep (Shrivastava and Talapatra, 1962a), swine (McCarthy et al., 1974) and chickens (Vogtmann et al., 1975). On the basis of the present study any one of the three AIA methods could be used to determine the digestibility of the feeds with similar accuracy.

When the 2N HCl method was compared to the total collection method in a digestibility study using sheep and ruminant feedstuffs (Experiment 2.), the values obtained by the 2N HCl method showed large variations in estimated alfalfa digestibility. These variations occurred because of day to day variation in AIA content of the alfalfa ration used. Furthermore, the alfalfa, having a low AIA value,

was not satisfactorily premixed before the experiment started. However, it appeared that the AIA levels in alfalfa samples obtained from the same bag remained constant. Therefore, if the alfalfa was mixed before the experiment, AIA variation could have been minimized. Similar results were obtained when estimates of digestibility from the 2N HCl method were compared with those from the total collection method (Study C).

When the AIA method is employed to estimate digestibility, as with other methods using an index substance, it is essential to obtain a representative sample of the feed ingested. Because the estimated level of index substance taken in by the animal is determined by that sample. Assessment of the composition of ingesta is generally not a problem for animals in confinement and offered a total ration. However, for grazing or free ranging animals or where a choice of feedstuffs is allowed it is more difficult to obtain a representative sample of the material consumed. The use of esophageally fistulated animals (Cook et al., 1963) may provide a useful means of obtaining ingesta samples under some circumstances. At times animals may consume feedstuffs contaminated by soil or other foreign material. Such contamination of ingesta would, if an appropiate correction could not be applied, result in a greater bias when AIA was used as an index substance than for other digestibility methods.

Accuracy of AIA method is poorest for feedstuffs, such

as alfalfa, with a low AIA content. With such materials extra care must be taken to ensure constancy of the ration fed and precision of analysis. Sampling and analysis of feces appears to be less of a problem because of its higher AIA content.

The AIA content of feces is estimated by chemical analysis of an average fecal sample. Therefore, a total collection of all the fecal material is not required. Care, however, must be exercised if samples are obtained off the ground to avoid contamination with soil, dust or bedding material. Problems of contamination can be avoided when samples of feces are obtained from the rectum (grab sample). When the AIA method is used for ration digestibility studies it offers some distinct advantages over other methods using index substances and the traditional total collection method. There is no need for special crates to confine or restrain the animals over an extended period of time for collection of feces. An estimate of feed digestibility can be obtained from an aliquot of feed intake and fecal output. Collecting fecal samples can be done at any time of the day (III. J.) and AIA occurs in common feedstuffs (III. G.) at readily measurable levels and laboratory procedures are not difficult nor time consuming. Furthermore, it is convenient to make repeated measurements on the same animal or on a relatively large number of animals. Thus statistically a loss in accurracy of a single measurement can be made up by increasing the number of measurements.

Possible Problems Pertaining to the AIA Method

During the employment of the AIA method in animal feeding trials it became obvious that feed and to a lesser extent fecal samples had to be collected with care. This was especially true when feeds with a low AIA content, such as alfalfa, were involved. Varying AIA levels in the alfalfa ration led to digestibility estimates that were unacceptable.

Collection of representative fecal samples appeared to be less troublesome. The higher AIA values of feces and the absence of diurnal variation in AIA excretion were an advantage. However, contamination of the feces by dust or other foreign materials, especially those containing high AIA content (soil) should be watched closely.

It should also be noted that the accuracy of assessing digestibility by the AIA method was based on data obtained by the total collection method, however, the latter method is by no means fool-proof. Errors made during a total collection trial are not easily detected. For example, fecal losses during a total collection period are difficult to estimate and their effect on ration digestibility goes unnoticed unless major variations occur. Calculations showing such possible errors were made by Petry and Enders (1974) when they compared the total collection method with the indicator method.

When tests were done to estimate the chemical composition of AIA it showed that approximately 99% of the

AIA was silica. It is possible that the AIA method only measures the insoluble components. The acid soluble silicas are removed during the analysis. Plants take up different amounts of silica according to their species and it is generally accepted that Gramineae contain 10 to 20 times the concentrations of silica found in legumes and other dicotyledons (Russel, 1961, p. 536). Total silica in the oat plant increased with increasing age, both in the tops as a whole (Jones and Handreck, 1965b) and in the parts (Handreck and Jones, 1968). Similar increases in total silica were observed in wheat (Russel, 1961).

The concentration of silica among various parts of a plant is different. For example, the glumes, paleae and lemmas of various cereals contain much higher concentrations of silica than the vegetative parts (Jones et al., 1963). This relationship was also shown in a wheat crop under field conditions (Hutton and Norrish, 1974). In oats the leaf blade contained 5.30% silica which represents 28% of the total silica in the plant and the silica content in the roots of crimson clover was about eight times that of the corresponding tops (Handreck and Jones, 1967). The lower concentration of total silica in the tops of legumes and other cotyledons was attributed to an exclusion of monosilicic acid from the transpiration stream, either within the root or at its external surface and there is evidence which suggest that metabolic processes are involved in each exclusion (Jones and Handreck, 1969). On the basis

of these observations it became clear that the AIA (being 99% silica) content of some rations (such as alfalfa) was subject to greater variation than the AIA content of some other rations.

Possible Applications

The AIA method offers a distinct advantage, as compared to the total collection method when used in an animal feeding trial, because the requirements for equipment and labor are reduced. Trials involving a greater number of animals and animals in non-experimental situations are possible since individual confinement is not necessary.

Since samples of feed and feces can be collected all within one day, an AIA analysis can start immediately and results made available within a three day period. At present, tests for feed digestibility at feed testing laboratories are rather limited. Long term feeding experiments are usually impractical. Data obtained through such a feeding trial is of reduced use to a farmer or feed mill, because of the time delay in obtaining information. The simplicity and convenience of the AIA method opens the possibility of its adaptation by feed testing laboratories responsible for evaluation of feedstuffs for animals in a practicle commercial environment.

BIBLIOGRAPHY

Association of Official Agricultural Chemists (AOAC). 1975. Official Methods of Analysis (12th Ed.). Washington, D.C.

Bailey, C.B. 1967. Silica excretion in cattle fed a ration predisposing to silica urolithiasis: total excretion and diurnal variations. Amer. J. Vet. Res. 28:1743.

Bailey, C.B. 1976. Fate of the silica in prairie hay and alfalfa hay consumed by cattle. Can. J. Anim. Sci. 56:213.

Blaxter, K.L. and H.H. Mitchell. 1948. The factorization of the protein requirements of ruminants and of the protein values of feed, with particular reference to the significance of the metabolic fecal nitrogen. J. Anim. Sci. 7:351.

Bondi, H. and H. Meyer. 1948. Lignin in young plants. Biochem. J. 43:248.

Brisson, G.J., W.J. Pigden and P.E. Sylvester. 1957. Effect of frequency of administration of chromic oxide on its fecal excretion pattern by grazing steers. Can. J. Anim. Sci. 37:90.

Carew, L.B. 1973. Establishing standardized procedures for metabolizable energy determination. Minneap. 19:25.

Chandler, P.T., E.M. Kesler and R.D. McCarthy. 1964. Polyethylene as a reference substance for digestion studies with young ruminants. J. Dairy Sci. 47:1426.

Chandler, P.T., E.M. Kesler and G.M. Jones. 1966. Excretion of polyethylene by dairy calves. J. Anim. Sci. 25:64.

Christopherson, R.J. 1976. Effects of prolonged cold and the outdoor winter environment on apparent digestibility in sheep and cattle. Can. J. Anim. Sci. 56:201.

Cook, C.W. and L.E. Harris. 1951. A comparison of the lignin ratio technique and the chromogen method of determining digestibility and forage consumption of desert range plants by sheep. J. Anim. Sci. 10:565.

Cook, C.W. J.T. Blake and J.W. Call. 1963. Use of esophageal-fistula cannulae for collecting forage samples from both sheep and cattle grazing in common. J. Anim. Sci. 22:579.

Corbett, J.L., T.B. Miller, E.W. Clarke and E. Florence. 1956. The use of polyethylene glycol as an inert reference substance for the estimation of fecal output. Proc. Nutr. Soc. 15. v.

Corbett, J.L., J.F.D. Greenhalgh and A.P. McDonald. 1958. Paper as a carrier of chromium sesquioxide. Nature. 182:1014.

Crampton. 1950. Problems, techniques and experimental design in animal investigations: Nutrition and pastures. J. Anim. Sci. 9:462.

Davis, C.L., J.H. Byers and L.E. Luber. 1958. An evaluation of the chromic oxide method for determining digestibility. J. Dairy Sci. 41:152.

Elam, C.J., P.A. Putnam and R.E. Davis. 1959. Fecal excretion pattern of chromium oxide administered to Hereford heifers in a completely pelleted ration. J. Anim. Sci. 18:719.

Ellenberger, H.B. and B.H. Schneider. 1927. Exercise as a factor in digestion trials with dairy cows. Vermont Agr. Exp. Sta. Bull. 262.

Ellis, G.H., G. Matrone and L.A. Maynard. 1946. A 72% H_2SO_4 method for the determination of lignin and its use in animal nutrition studies. J. Anim. Sci. 5:285.

Engelhardt, W.V., G. Becker, W. Engelhardt, R. Hauffe, S. Hinderer, K. Rubsamen and W. Schneider. 1974. ——— . In: Tracer techniques in tropical animal production, pp. 111-124. International Atomic Energy Agency, Vienna.

Fletcher, H.F. and L.T. Kuntz. 1964. Differential Effects of phosphorus fertility on soybean varieties. Soil Sci. Soc. Am. Proc. 28:225.

Forman, S.A. and F. Sauer. 1962. Some changes in the urine of sheep fed a hay high in silica. Can. J. Anim. Sci. 42:9.

Gallup, W.D. 1929. A note on the determination of the digestibility of protein by Bergheim's method. J. Biol. Chem. 76:43.

Gallup, W.D. and A.H. Kuhlman. 1931. A preliminary study of the apparent digestibility of protein by modified procedures. J. Agr. Res. 42:665.

Gallup, W.D. and A.H. Kuhlman. 1936. The composition and digestibility of mungbean silage, with observations on the silica-ratio procedure for studying digestibility. J. Agr. Res. 52:889.

Gallup, W.D., C.S. Hobbs and H.M. Briggs. 1945. The use of silica as a reference substance in digestion trials with ruminants. J. Anim. Sci. 4:68.

Garrigus, W.P. 1934. The forage consumption of grazing steers. Proc. Am. Soc. An. Prod. p.66.

Greenhalgh, J.F.D. and J.L. Corbett. 1960. The indirect estimation of the digestibility of pasture herbage. I. Nitrogen and chromogen as fecal index substances. J. Agr. Sci. 55:371.

Grubb, J.A. and B.A. Dehority. 1975. Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in scheep. J. Appl. Bacteriol. 30:404.

Handreck, K.A. and L.H.P. Jones. 1967. Uptake of monosilicic acid by *Trifolium Incarnatum* (L.). Austr. J. Biol. Sci. 20:483.

Handreck, K.A. and L.H.P. Jones. 1968. Studies of silica in the oat plant. IV. Silica content of plant parts in relation to stage of growth, supply of silica and transpiration. Plant and Soil. 24:449.

Haurowitz, F. 1963. The chemistry and function of proteins. 2nd. Ed. Academic Press, New York. p.26.

Holter, J.A. and J.T. Reid. 1959. Relationship between the concentration of crude protein and apparently digestible protein in forages. J. Anim. Sci. 18:1339.

Hungate, R.E. 1957. Microorganisms in the rumen of cattle fed a constant ration. Can. J. Microbiol. 3:289.

Hungate, R.E. 1966. The rumen and its microbes. Academic Press, New York.

Hutton, J.T. and K. Norrish. 1974. Silicon content of wheat husks in relation to water transpired. Austr. J. Agr. Res. 25:203.

Jones, L.H.P., A.A. Milne and S.M. Wadham. 1963. Studies of silica in the oat plant. II. Distribution of the silica in the plant. Plant and Soil. 18:358.

Jones, L.H.P. and K.A. Handreck. 1965a. The relation between the silica content of the diet and the excretion of silica by sheep. J. Agr. Sci. 65:129.

Jones, L.H.P. and K.A. Handreck. 1965b. Studies of silica in the oat plant. III. Uptake of silica from soils by the plant. Plant and Soil. 23:79.

Jones, L.H.P. and K.A. Handreck. 1969. Uptake of silica by *Trifolium incarnatum* in relation to the concentration in the external solution and to transpiration. Plant and Soil. 30:71.

Kane, E.A., R.E. Ely, W.C. Jacobson and L.A. Moore. 1951. Comparitive digestion studies on orchard grass. J. Dairy Sci. 34:492.

Kane, E.A., W.C. Jacobson, R.E. Ely and L.A. Moore. 1953. The estimation of the dry matter consumption of grazing animals by ratio technique. J. Dairy Sci. 36:637.

Kiesling, H.E., H.A. Barry, A.B. Nelson and C.H. Herbel. 1969. Recovery of chromic oxide administered in paper to grazing animals. J. Anim. Sci. 29:361.

Kleiber, M. 1975. The fire of life. Robert and Kreiger Publ. Comp., Huntington, New York. p.260.

Kotb, A.R. and T.D. Luckey. 1972. Markers in nutrition. Nutr. Abstr. Rev. 42:813.

Lancaster, R.J. 1949. Estimation of digestibility of grazed pasture from feces nitrogen. Nature 163:330.

Lancaster, R.J. 1954. Measurement of feed intake of grazing cattle and sheep. V. Estimation of the feed-to-feces ratio from the nitrogen content of the feces of pasture fed cattle. New Zealand J. Sci. Tech. A.36:15.

Langlands, J.P. 1975. Techniques for estimating nutrient intake and its utilization by the grazing animal. In: Digestion and metabolism in the ruminant. pp. 320-332. Eds. I.W. McDonald and A.C.I. Warner. Proc. of the IV Int. Symp. on Ruminant Physiol. The Univ. of New England Pub. Unit.

MacRae, J.C. 1974. The use of intestinal markers to measure digestive function in ruminants. Proc. Nutr. Soc. 33:147.

McCarthy, J.F., F.X. Aherne and D.B. Okai. 1974. Use of HCL insoluble ash as an index material for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54:107.

McDonald, I.W. 1968. The nutrition of grazing ruminants. Nutr. Abstr. Rev. 38:881.

McDonald, P., R.A. Edwards and J.F.D. Greenhalgh. 1969. Animal nutrition. p.144. Oliver and Boyd Ltd. Edinburgh, England.

Minson, D.J. and C.D. Kemp. 1961. Studies in the digestibility of herbage. IX. Herbage and fecal nitrogen as indicators of herbage organic matter digestibility. J. Brit. Grassl. Soc. 16:76.

National Academy of Sciences. 1968. Nutrient requirements of sheep. Publication No. 1693. Washington, D.C.

Nicholson, J.W., E.H. Haynes, R.G. Warner and J.K. Loosli. 1956. Digestibility of various rations by steers as influenced by the length of preliminary feeding period. J. Anim. Sci. 15:1172.

Noblitt, C.G., W.A. Hardison, J.T. Huber and G.C. Graf. 1963. Effect of manual total collection of feces upon nutrient digestibilities. J. Dairy Sci. 46:67.

Nottle, M.C. 1966. Silica metabolism of the Merino sheep. Austr. J. Agr. Res. 17:175.

Nottle, M.C. and J.M. Armstrong. 1966. Urinary excretion of silica by grazing sheep. Austr. J. Agr. Res. 17:165.

Okuda, A. and E. Takahashi. 1964. ———. In "The mineral nutrition of the rice plant", Symp. Intern. Rice Res. Inst., pp.123-146. John Hopkins Press, Baltimore, Maryland.

Parry, D.W. and F. Smithson. 1957. Detection of opaline silica in grass leaves. Nature. 179:975.

Petry, H. and H. Enders. 1974. Critical considerations on the reliability coefficients determined by conventional total fecal collection method and chromium (III) oxide indicator method. Z. Tierphysiol., Tierernahr. Futtermittelk. 33:88.

Phar. P.A., N.W. Bradley, C.O. Little, L.V. Cundiff and J.A. Boling. 1971. Nutrient digestibility using fecal collection apparatus and indicator method for steers fed ad libitum. J. Anim. Sci. 33:695.

Pigden, W.J. and J.G. Brisson. 1957. Note on a chromium oxide pellet to provide release of this indicator in the rumen of cattle. Can. J. Anim. Sci. 37:185.

Raymond, W.F. 1951. The problem of measuring the nutritive value of herbage. J. Brit. Grassl. Soc. 6:139.

Raymond, W.F., C.D. Kemp, A.W.Kemp and C.E. Harris. 1954. Studies in the digestibilities of herbage. IV. The use of fecal collection and chemical analysis in pasture studies (b) Fecal index methods. J. Brit. Grassl. Soc. 9:69.

Raymond, W.F. 1969. The nutritive value of forage crops. Adv. in Agron. 21.1.

Reid, T.J. P.G. Woolfolk, C.R. Richards, J.I. Miller and R.E. Blaser. 1950. A new indicator method for determination of digestibility and consumption of forages by ruminants. J. Dairy Sci. 33:60.

Reid, J.T., P.G. Woolfolk, W.A. Hardison, C.M. Martin, A.L. Brundage and R.W. Kaufmann. 1952. A procedure for measuring the digestibility of pasture forage under grazing conditions. J. Nutr. 46:255.

Richards, C.R. and J.T. Reid. 1952. The use of materials as an index of the feeding value of forages. J. Dairy Sci. 35:595.

Russel, E.W. 1961. Soil conditions and plant growth. 9th Ed. Longmanns Green, New York.

Schneider, B.H., B.K. Soni and W.E. Ham. 1955. Methods for determining consumption and digestibility of pasture forages by sheep. Wash. Agr. Exp. Sta. Tech. Bull. 16.

Schneider, B.H. and W.P. Flatt. 1975. The evaluation of feeds through digestibility experiments. The Univ. of Georgia Press, Athens. U.S.A.

Shrivastava, V.S. and S.K. Talapatra. 1962a. Pasture studies in Uttar Pradesh. II use of some natural indicators to determine the plane of nutrition of a grazing animal. Indian J. Dairy Sci. 15:154.

Shrivastava, V.S. and S.K. Talapatra. 1962b. Pasture studies in Uttar Pradesh. III Determination of pasture consumption by grazing animals. Indian J. Dairy Sci. 15:161.

Sinha, K.N., F.A. Martz, H.D. Johnson and LeRoy Hahn. 1970. Use of polyethylene glycol in estimating volume and weight of rumen contents and digestion in cattle. J. Anim. Sci. 30:467.

Smith, A.M. and J.T. Reid. 1955. Use of chromic oxide as an indicator of fecal output for the purpose of determining the intake of pasture herbage by grazing cows. J. Dairy Sci. 38:515.

Smith, A.D., R.B. Turner and G.E. Harris. 1956. The apparent digestibility of lignin by mule deer. J. Range Manage. 9:142.

Smithson, F. 1958. Grass opal in British soils. J. Soil Sci. 9:148.

Sperber, I., S. Hyden and J. Ekman. 1953. The use of polyethylene glycol as a reference substance in the study of ruminant digestion. Ann. Agr. Coll. Sweden. 20:337.

Steel, R.C.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., Toronto, Ontario.

Streeter, C.L. 1969. A review of techniques used to estimate the *in vivo* digestibility of grazed forage. J. Anim. Sci. 29:757.

Tullin, V. 1954. Response of the sugar beet to common salt. Physiol. Plantarum. 7:810.

Van Dyne, G.M. and J.H. Meyer. 1964. Forage intake by cattle and sheep on dry annual range. J. Anim. Sci. 23:1108.

Van Dyne, G.M. and D.T. Torrell. 1964. Development and Use of esophageal fistula: A review. J. Range Manage. 17:7.

Van Soest, P.J. 1964. Symposium on nutrition and forage and pasture: New chemical procedures for estimating forages. J. Anim. Sci. 23:838.

Van Soest, J. 1969. Newer knowledge on the composition and methods of analysis of feeding stuffs (Chapter 2). Nutrition of animals of agricultural importance. Part 1. The science of nutrition of farm livestock. D. Cuthbertson ed. Pergamon Press Ltd., Oxford.

Vogtmann, H., H.P. Pfirter and A.L. Prabucki. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Brit. Poul. Sci. 16:531.

Waldo, D.R., C.E. Coppack, L.W. Smith, L.A. Moore and J.W. Sykes. 1961. Annual report of S-45 Regional Committee on the Nutrient evaluation of forage crops. U.S.D.A., Wash. D.C.

Warner, A.C.I. 1962. Some factors influencing the rumen microbial population. J. Gen. Microbiol. 28:129.

Weisenburger, R.D. 1976. Straw utilization by beef cattle M.Sc. thesis. Univ. of Alberta. Edmonton, Alberta. Canada.

Westra, R. 1975. The effect of temperature on digestion in sheep. M.Sc. thesis, Univ. of Alberta, Edmonton, Alberta, Canada.

APPENDIX 1

PROCEDURE FOR THE DETERMINATION OF AIA BY THE CONC. HCL METHOD

Ref: Shrivastava, V.S. and S.K. Talapatra, 1962. Pasture studies in Uttar Pradesh. II. Use of some natural indicators to determine the plane of nutrition of a grazing animal. Indian J. Dairy Sci. 15:154.

Reagents:

1. An aqueous, concentrated HCl solution.
2. Ashless filter paper, Whatman No 41.

Procedure:

1. Weigh initial prepared sample (dried-ground), 10 g of feed or 20 g of feces, into wide crucible (150 ml), dry for 2 hours at 135 C in a forced-air oven, cool crucible in desiccator to room temperature and weigh crucible with dry matter.

2. Ash crucible with sample over a five hour period by progressively increasing the furnace temperature from 250 to 650 C in 100 C increments.

3. Moisten the resulting ash with 5 ml of water, and 10 ml of concentrated HCl and evaporate to dryness over a waterbath. Repeat this step twice.

4. Add 5 ml of concentrated HCl to the sample residue and after 15 min over the waterbath filter the contents of the crucible. Wash sample free of acid with hot distilled water, transfer filter paper with ash to crucible and ash overnight at 650 C.

5. Cool crucible in desiccator to room temperature, weigh crucible with ash and weigh crucible immediately after emptying.

Calculations:

$$\% \text{ Conc. HCl insouble ash} = \frac{(Wf - We) \times 100}{Ws}$$

Wf = weight of crucible with ash, in g.
We = weight of empty crucible, in g.
Ws = weight of sample; in g dry matter.

APPENDIX 2

PROCEDURE FOR THE DETERMINATION OF AIA BY THE 4N HCl METHOD

Ref: Vogtmann, H., H.P. Pfirter and A.L. Prabuki. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Brit. Poul. Sci. 16:531.

Reagents:

1. Aqueous 4N HCl solution.
2. Ashless filter paper, Whatman No 41.

Procedure:

1. Weigh accurately 10 to 12 g of well mixed and fine ground sample into 800 ml Erlemeyer flask. Add 100 ml aqueous 4N HCl solution and boil gently for 30 minutes, eventually with occasional swirling to prevent bumping.

2. Filter the hot hydrolysate through an ashless filter paper, washing the beaker several times with hot distilled water and transfer this to the filter paper. Wash sample free of acid (pH 6) with hot distilled water (filterate will be nearly colorless) and discard filtrate.

3. Transfer filter paper, containing the sample, carefully into a weighed crucible and ash at approximately 650 C overnight (minimum 6 hours).

4. Cool crucible in desiccator to room temperature and determine the weight of the ash.

Calculations:

$$\% \text{ 4N HCl insoluble ash} = \frac{(Wf - We) \times 100}{Ws}$$

Wf = weight of crucible with ash, in g.
We = weight of empty crucible, in g.
Ws = weight of sample, in g dry matter.

APPENDIX 3

PROCEDURE FOR THE DETERMINATION OF AIA BY THE 2N HCl METHOD

Reagents:

1. Aqueous 2N HCl solution.
2. Ashless filter paper, Whatman No 41, diameter 18 cm.

Procedure:

1. Initial preparation of sample.
    a. Drying
    b. Grinding

2. a. Weigh accurately 5.0 g samples (to 4 decimal places into crucible (50 ml).
    b. Dry for 2 hours at 135 C (forced air oven).
    c. Cool crucible in desiccator ($CaSO_4$) to room temperature.
    d. Weigh crucible with dry matter.

3. Ash crucible with sample overnight at 450 C (± 25 C).

4. a. Transfer ash into 600-800 ml Berzelius beaker.
    b. Add 100 ml aqueous 2N HCl and boil gently for 5 minutes.
    c. Filter the hot hydrolysate through an ashless filter paper.
    d. Wash sample free of acid with hot distilled water, (300 ml) and discard filtrate.
    e. Transfer filter paper with ash into crucible.

5. Ash crucible overnight at 450 C (± 25 C).

6. a. Cool crucible in desiccator to room temperature.
    b. Weigh crucible with ash. ⟶ % DM
    c. Weigh empty crucible. ⟶ % Acid-insoluble ash.

Calculations:

$$\text{\% 2N HCl insouble ash} = \frac{(Wf - We) \times 100}{Ws}$$

Wf = weight of crucible with ash, in g.
We = weight of empty crucible in g.
Ws = weight of sample in g dry matter.